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Development of A Rapid and Sensitive LC-MS Method for Determination of Aprepitant Levels in the Plasma of Subacute Sclerosing Panencephalitis Patients

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Subakut Sklerozan Panensefalitli Hastaların Plazmasında Aprepitant Tayini İçin Hassas ve Hızlı Bir LC-MS Yönteminin Geliştirilmesi

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Abstract

In this study, a sensitive, selective, rapid, and validated Liquid Chromatography-Mass Spectrometry (LC-MS) method was developed for routine monitoring of aprepitant concentrations in the blood of patients with subacute sclerosing panencephalitis (SSPE). The developed method was effectively used to detect and monitor potential drug-induced toxicity in SSPE patients. The analyte was extracted from human plasma using liquid-liquid extraction. Chromatographic separation was carried out on a C18 (50 mm x 2.1 mm x 3 µm) column with acetonitrile/0.2% formic acid- water (50/50, v/v)) isocratic mobile phase at a flow rate of 0.40 mL/min. Mass spectrometry analysis was performed in positive mode using the electrospray ionization technique, and aprepitant was detected at 535.20 m/z.The retention time for the aprepitant was 2.70 min, and the total retention time was 5 min. The linear concentration range was 0.0125-3.00 µg/mL (r=0.9976), LOD and LOQ values were 0.010 µg/mL and 0.030 µg/mL, respectively. The developed method was successfully used for the determination of aprepitant concentration in plasma samples from SSPE patients. The developed and validated method is selective, sensitive and rapid, making it suitable for routine use for aprepitant detection in preclinical and clinical studies.

Keywords: *Aprepitant; Determination; LC-MS; Subacute Sclerosing Panencephalitis; Human plasma.*

Öz

Bu çalışmada, Subakut Sklerozan Panensefalit (SSPE) hastalarının kanındaki aprepitant düzeylerinin rutin olarak izlenmesi için hassas, seçici, hızlı ve doğrulanmış bir Sıvı Kromatografisi-Kütle Spektrometrisi (LC-MS) yöntemi geliştirilmiştir. Geliştirilen bu yöntem, SSPE hastalarında ilaca bağlı potansiyel toksisitenin tespit edilmesi ve izlenmesinde etkili bir şekilde kullanılmıştır. Aprepitant, insan plazmasından sıvı-sıvı ekstraksiyonu yöntemi kullanılarak ekstrakta edilmiştir. Kromatografik analiz için en uygun koşullar, C18 (50 mm x 2,1 mm x 3 µm) kolonu, asetonitril/su (50/50 (%0,2 formik asit) v/v) izokrotik mobil fazı ve 0,40 mL/dakika akış hızı olarak bulunmuştur. Kütle spektrometresinde, pozitif modda, elektrosprey iyonizasyon tekniği ile çalışılmış ve aprepitant 535.20 m/z değerinde tespit edilmiştir. Aprepitant için alıkonma zamanı 2,70 dakika, toplam analiz süresi 5 dakika, doğrusal konsantrasyon aralığı 0,0125-3,00 µg/mL (r=0,9976), LOD ve LOQ değerleri sırasıyla 0,010 µg/mL ve 0,030 µg/mL olarak bulunmuştur. Geliştirilen yöntem, SSPE hastalarından alınan plazma örneklerinde aprepitantın konsantrasyonunun tespiti için başarıyla kullanılmıştır. Geliştirilen ve valide edilen bu yöntem seçici, duyarlı ve hızlı bir yöntemdir. Preklinik ve klinik çalışmalarda, aprepitant tespiti için güvenle ve rutin olarak kullanılabilir.

Anahtar Kelimeler: *Aprepitant; Miktar tayini; LC-MS; Subakut Sklerozan Panensefalit; İnsan plazması*

1. Introduction

Aprepitant (Figure 1), a neurokinin-1 (NK-1) receptor antagonist, was approved by the FDA in September 2015 for the treatment of chemotherapy-induced nausea and vomiting (CINV) (Adel 2017). The aprepitant regimen for adults is scheduled as 125 mg/day on the first day of chemotherapy and 80 mg/day on the second and third days (Hesketh et al. 2003, Manak et al. 2010).

Recent in-vitro and in-vivo studies have shown that Substance P and NK-1 receptors have antiviral properties against various viral diseases, such as Human Immunodeficiency Virus (HIV) infection (Hesketh et al.

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2003, Manak et al. 2010, Safwat et al. 2023, Mehboob et al. 2021), herpes virus infection (Twardy et al. 2011, Makhortova et al. 2007) and measles virus (MV) infection (Makhortova et al. 2007).

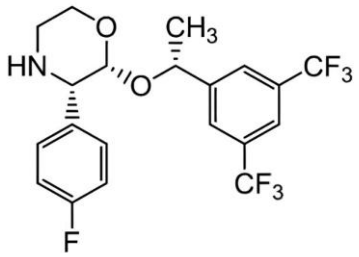

Figure 1. Chemical structure of aprepitant

A recent review emphasized that NK-1 receptor antagonists have potential antiviral effects, and further investigation is needed to explore their potential therapeutic antiviral effects (Wenya et al. 2018). Ongoing in vitro and in vivo studies are investigating the clinical application of aprepitant as an antiviral drug (Tebas et al. 2011, Tebas et al. 2015). Subacute Sclerosing Panencephalitis (SSPE) is a severe and often fatal complication of measles virus infection, manifesting neurological symptoms after a latent period of 2-10 years following the primary measles infection, with no specific treatment currently available for the measles virus itself (Jafri et al. 2018, Samia et al. 2022, Oncel et al. 2020). In the first clinical trial examining aprepitant treatment for SSPE patients, it was reported that aprepitant was safe, well-tolerated, and did not cause any serious side effects (Oncel et al. 2020). Several methods for aprepitant determination have been reported in the literature. Erdoğar et al. (2021) developed a validated LC-MS-MS method for the analysis of aprepitant from rat plasma (Erdoğan et al. 2021). Chavez-Eng et al. developed an HPLC-tandem mass spectrometry method using chemical ionisation in positive mode at m/z 535/277 (Chavez et al. 2004). PVDLS et al. (2013) developed an LC-MS/MS-MRM (multiple reaction monitoring) method for the determination of aprepitant at 535.10/277 m/z (PVDLS et al. 2013). Chaitanya et al. (2013) reported an HPLC and ion-trap mass spectrophotometry method with a positive ion mode at m/z 535 (Chaitanya et al. 2013). Nijstad et al. (2021) developed a sensitive LC-MS/MS method for the simultaneous quantification of aprepitant in small volumes of paediatric human plasma (Nijstad et al. 2021). Naito et al. (2021) developed an LC-MS method utilizing electrospray ionization (ESI) for quantifying total plasma and free aprepitant, as well as its N-dealkylated metabolites in human plasma (Naito et al. 2021).

This study is part of a clinical trial aimed at developing a novel treatment with aprepitant (APT) for SSPE patients (Oncel et al. 2020). In this study, a simple, reliable, selective, sensitive, and rapid LC-MS method was developed using a small plasma volume of just 0.25 mL. The validated method was employed to measure aprepitant plasma concentrations and monitor drug levels in SSPE patients.

2. Materials and Methods

2.1 Chemicals and reagents

Aprepitant was received from the Merck (Rahway, NJ, USA). HPLC-grade acetonitrile, formic acid, and methanol were purchased from Merck (Rahway, NJ, USA). The water was obtained from the purification system Synergy UV (Millipore, Milli-Q-RG, Darmstadt, Germany).

2.2. LC-MS instrumentation

The LC-MS system comprised a single quadrupole mass spectrometer (Shimadzu 2020, Japan) with an electrospray ionization (ESI) source and a pump and autosampler system. The mass spectrometer conditions using the ESI source were optimized by operating in both positive and negative modes, adjusting nebulization gas, drying gas, Quarry RD, and Quarry DC. Separations were carried out using a C18 analytical column (Athena 50 mm x 2.1 mm x 3 µm).

2.3. Stock, standard solutions, and sample preparation

The primary stock solutions of aprepitant were prepared in acetonitrile (1.00 mg/mL). From this stock solution, dilutions (0.10 and 0.010 mg/mL) were made with acetonitrile. The stock solutions of aprepitant were stored at +4ºC and were found to be stable for at least 24 hours (Wu *et al.* 2020). Nine different concentrations of aprepitant (0.0125-3.00 µg/mL) solutions for calibration curves were added to 0.25 mL blank plasma, and 1.0 mL cold acetonitrile was added into the Eppendorf tubes. Then, the mixture was vortexed for 5 min and centrifuged for 15 min at 3000 rpm at +4ºC. The supernatant was filtered through the 0.45 µm membrane filter and collected in the vials. Then, 10 µL of this solution was injected into the LC-MS system. An internal standard was not used in this study. This decision was based on the observation that plasma matrix components do not interfere with the peak retention time of aprepitant. This result shows that the method is selective. Furthermore, reproducibility studies confirmed that the analytical results were within statistically acceptable limits without the use of an internal standard. These observations are in agreement with some studies (Imre *et al.* 2019, Özçelik *et al.* 2023, Kara *et al.* 2023) showing that in certain situations (especially where matrix effects are minimal and method reproducibility is robust) internal standards may not be necessary for reliable quantification.

2.4. Quantification of aprepitant in human plasma

The study received ethical approval from the Clinical Research Ethics Committee of Keçiören Training and Research Hospital (2012 KAEK-15), under the protocol code 2014-AKD-30. Ten patients with SSPE were enrolled in the study. All patients and/or their guardians provided written informed consent after receiving comprehensive information about the study design and protocol. SSPE patients were administered with 250 mg aprepitant orally once a day for 15 days (Oncel *et al.* 2020). Blood samples were collected on the first day of the treatment. Blood samples of patients with (1mL) were taken into the heparinized tubes before the administration of aprepitant (0. hour), at the 4th hour (time to reach peak plasma concentration) after the first dose, and finally at the 12th hour. The blood samples taken were kept in ice for 5-10 minutes, then they were centrifuged rapidly (3.000 rpm (1006 (× g) RCF), 15 minutes, +4ºC), and the separated plasma was kept until analysis at -20ºC. All solutions were prepared freshly before analysis. An aliquot of 0.25 mL plasma of the patients and 1.0 mL cold acetonitrile was added into the Eppendorf tubes. Then, the mixture was vortexed for 5 minutes and centrifuged for 15 minutes at 3000 rpm at +4ºC. The supernatant was filtered through the 0.45 µm membrane filter and collected in the vials. Then, 10 µL of this solution was injected into the LC-MS system.

3. Results

3.1 Method development

The developed chromatographic method was optimised on a reversed-phase C18 pre-column (Athena 20 mm x 4.0 mm x 5µm) and C18 column (Athena 50 mm x 2.1 mm, 3 µm) and the isocratic mobile phase mixture of acetonitrile/ 0.2% formic acid- water) (50/50, v/v). At room temperature, an injection volume of 10 µL was used and a flow rate of 0.40 mL/min was used. Reproducibility studies were found to be within statistically significant limits; therefore, no internal standard was used in this study. Shimadzu LC-MS 2020 and ESI technique were used and operated in a positive ion mode. The nebulizer gas flow was set to 1.5 L/min, and the drying gas flow was set to 15 L/min., and quarry RF and quarry DC values were set to 52 and -5, respectively. Analytical data were processed using Shimadzu LabSolution Software (ver. 5.123). During the direct infusion experiment, the mass spectrum for the aprepitant was determined as a protonated molecular ion $[M + H]^+$ at m/z 535.20.

3.2 System suitability

Under optimized analytical conditions, the system's suitability was assessed based on column efficiency (theoretical plate number, N), capacity factor (k), and tailing factor. The obtained values (N = 4653, k = 4.6, and tailing factor = 1.69) were within the specified limits, confirming that the system was suitable for analyzing APT in human plasma.

3.3 Validation study

The validation of this study was performed in accordance with FDA guidelines (Bioanalytical method validation 2018. Food and Drug Administration). To determine the quantitative performance of the developed analytical method, selectivity, specificity and matrix effect, linearity, limits of detection (LOD) and limits of quantification (LOQ), intra- and inter-day precision, and accuracy and recovery of analytes were tested.

3.3.1 Selectivity

The selectivity of the method was evaluated by comparing the chromatograms of blank human plasma and APT-spiked human plasma samples (Figure 2 and Figure 3). As shown in the figures, no interfering peak from the blank plasma was observed at the retention time of the APT peak, indicating that the method is selective for APT analysis in human plasma samples.

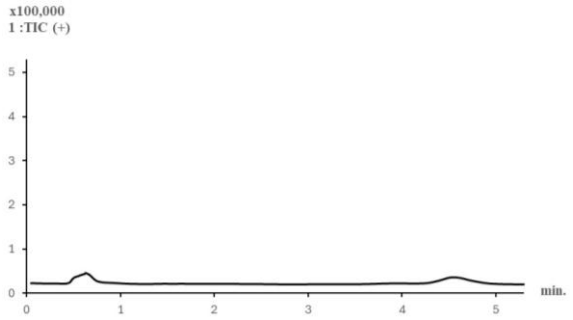

Figure 2. The chromatograms of the control human plasma (analyte-free)

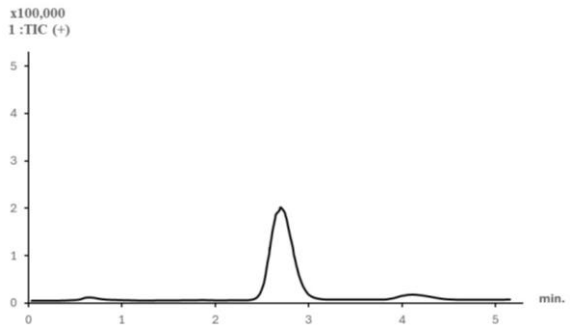

Figure 3. The Chromatogram of 0.50 µg/mL aprepitant in plasma

3.3.2 Matrix effect

For the matrix effect evaluation, the peak area values obtained in the presence and absence of the sample

matrix were compared. APT as added to both blank plasma and water at a concentration of 0.50 µg/ml. The samples were then analyzed using the LC-MS method developed in this study, and the APT peak areas in each group were compared. The matrix factor was found to be 101.72 ± 11.15, indicating that the matrix effect of the plasma samples is acceptable for the developed method.

3.3.3 Linearity

The calibration curve was prepared by working in a blank plasma. This curve was generated using nine different concentrations (0.0125-3.00 µg/mL). Correlation coefficient r=0.9976±0.002 (mean ± SE; n=3). The linear regression equations were found as y = 1454x + 2.72. This equation is the mean of the curves repeated at the same concentration ranges at different times.

3.3.4 Sensitivity

The detection limit (LOD) and quantification limit (LOQ) were identified as the concentration of APT corresponding to signal-to-noise ratios of 3:1 and 10:1, respectively. In this research, the LOD and LOQ values for APT were found to be 0.010 µg/mL and 0.030 µg/mL, respectively. The method exhibited a precision below 5.0% RSD at the LOQ level.

3.3.5 Accuracy and precision

Intraday and interday analyses were performed to find the accuracy and precision of the developed method. These analyses were performed at three concentration levels (0.10, 0.70, 3.00 µg/ml) within the linearity range of APT in plasma samples, with three repeated measurements on the same day and three consecutive days (Table 1). The results obtained from intraday and interday evaluations were within acceptable limits of variation.

Table 1. Accuracy and precision data for intra-day and inter-day in human plasma

Inter-day			Intra-day	
Theoretical C (µg/mL)	**Measured C(µg/mL) ± SD(n=3)**	**RSD %**	**Measured C (µg/mL) ± SD (n=3)**	**RSD %**
0.10	0.112±0.002	1.8	0.084±0.002	2.4
0.70	0.654±0.001	0.15	0.570±0.014	2.5
3.00	2.854±0.006	0.21	2.300±0.020	0.9

SD: standard deviation;C: concentration n: number of QC samples; RSD: relative standard deviation

3.3.6 Recovery

For the recovery test, APT was added to blank human plasma at different concentration levels (low: LQC 0.10 µg/mL, medium: MQC 0.70 µg/mL and high: HQC 3.00 µg/mL) and analysed by the developed method. The concentration values corresponding to the peak area values obtained as a result of the analysis were calculated using the calibration curve. Recovery rates were determined using the averages of three repeated measurements for each concentration and the results are presented in Table 2.

Table 2. Recovery of the method

Theoretical C (µg/mL)	Measured C (µg/mL) ± SD (n=3)	RSD %	Recovery %
0.10	0.099 ± 0.004	4.04	99.0
0.70	0.710 ± 0.036	5.08	101.4
3.00	2.870 ± 0.078	2.72	95.7

SD: standard deviation; C: concentration; n: number of QC samples; RSD: relative standard deviation

3.4 Robustness

The robustness of the developed method was evaluated by making small adjustments to the optimized parameters. Specifically, the percentage of FA in water (ranging from 0.019% to 0.21%), the proportion of organic solvent (acetonitrile) in the mobile phase (49% to 51%), and the flow rate (0.39 to 0.41 mL/min) were slightly varied. No significant statistical differences were observed between the results ($p \geq 0.05$). Thus, it can be concluded that these minor variations did not affect the APT peak area, confirming that the analytical method is robust.

3.5 Stability

To determine the stability of the method, APT standard solutions were analysed after 24 hours at room temperature, 12 hours at room temperature in plasma solutions and autosampler and 30 days at -20^{0}C. The samples were found to be stable as the coefficient of variation was below the acceptable limit of ±15%.

3.6 Aprepitant plasma levels of the patients

The aprepitant plasma levels of 16 SSPE patients were measured on the first day of the therapy, and the results were published in a recent article (Oncel *et al.* 2020). The maximum plasma concentration (Cmax) for the aprepitant was found at the 4th and 12th hours as 0.81± 0.17 µg/mL and 0.45± 0.21, respectively (Oncel *et al.* 2020).

4. Discussion

The determination of plasma levels of certain drugs is very crucial to provide therapeutic drug monitoring or to discover the new effects of the drugs. Particularly, the COVID-19 outbreak presents many drugs for other

indications that are used to treat the disease (Mehboob *et al.* 2021, Schirinzi *et al.* 2023). A recent clinical trial was conducted to assess the efficacy and safety of aprepitant injectable emulsion when added to the standard of care for hospitalized COVID-19 patients (Schirinzi *et al.* 2023). There is no standardised method for therapeutic drug monitoring of aprepitant as an antiemetic drug. Validated analysis methods are very essential for preclinical and clinical studies. For this purpose, an LC-MS method for the determination of aprepitant in human plasma was developed in this study. The use of internal standards was not required in this study. The results of the study, e.g. the absence of plasma matrix interference at the APT retention time, demonstrated the selectivity of the method, while the robustness of the calibration curves and the precision of intra- and inter-day analyses supported the reproducibility of the method. In a study by Imre et al. it was shown that analytical accuracy and precision can be achieved without the use of internal standards, provided that the method has high reproducibility (Imre *et al.* 2019). There are also studies in the literature reporting successful LC-MS-MS analyses without the use of internal standards due to matrix effects and strong method robustness (Özçelik *et al.* 2023, Kara *et al.* 2023).

This method, developed for the use of aprepitant in preclinical and clinical studies, is also very simple in terms of both sample preparation and analysis. In this study, aprepitant was extracted from plasma by liquid-liquid extraction technique using acetonitrile. A small plasma volume of 0.25 mL was used, which is very important since patients with rare diseases were studied. The analysis was completed in as little as 5 minutes. Therefore, this method is a fast method. No interference from plasma components was observed, demonstrating the selectivity of the method. The low maximum column loading ensured optimal column efficiency for analyzing plasma samples. The developed and validated method is a rapid, simple, and selective approach that can be routinely used for aprepitant determination in both clinical and preclinical studies.

5. Conclusions

In this study, a precise, reliable, and reproducible LC-MS method for determining aprepitant in plasma was developed and validated. The method was successfully applied to monitor aprepitant plasma levels in a clinical study with SSPE patients, demonstrating its effectiveness. The validated method is suitable for routine analysis of aprepitant in drug formulations and biological samples.

Declaration of Ethical Standards

* This article is based on a poster titled "Therapeutic Drug Monitoring of Aprepitant with Liquid Chromatography–Mass Spectrometry," which was presented at the 51st European Society of Clinical Pharmacy (ESCP) Symposium in Aberdeen, Scotland (31 October-2November 2023). While the full text of the poster was not previously published, the content has been further developed and partially revised for this publication.

The authors declare that they comply with all ethical standards. The study received ethical approval from the Clinical Research Ethics Committee of Keçiören Training and Research Hospital (2012 KAEK-15), under the protocol code 2014-AKD-30.

Credit Authorship Contribution Statement

Author-1: Methodology, Study design, Data curation, Software, validation, Formal analysis, Investigation, Writing-original draft, Writing–review and editing, Visualization, Project administration, Funding acquisition, Supervision.

Author-2: Study design, Software, Validation, Formal analysis, Investigation.

Author-3: Study design, Formal analysis, Data curation, Investigation, Writing – original draft, Writing – review and editing, Visualization.

Author-4: Study design, Formal analysis Investigation, Resources, Funding acquisition.

Author-5: Data curation, Software, Investigation, review and editing.

Author-6: Data curation, Software, Investigation, review and editing.

Author-7: Data curation, Software, Investigation, review and editing.

Author-8: Data curation, Project administration, Funding acquisition, Supervision. Writing–review and editing,

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability Statement

All data generated or analyzed during this study are included in this published article.

Datasets are available on request. The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

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