

COMPARATIVE, RANDOMIZED, SINGLE-DOSE, CROSSOVER BIOAVAILABILITY STUDY OF TWO FORMULATIONS OF AZITHROMYCIN 500 mg FILM-COATED TABLETS IN HEALTHY VOLUNTEERS UNDER FASTING CONDITIONS

Gordana Damjanovska², Emilija Atanasovska¹, Nikola Labacevski¹

¹Institute for preclinical and clinical pharmacology and toxicology, Faculty of Medicine,
“Ss.Cyril and Methodius University” in Skopje, North Macedonia

²Medical doctor, PhD, Specialist for preclinical and clinical pharmacology and toxicology,
Skopje, North Macedonia

Abstract

Bioequivalence studies are clinical studies for determining bioavailability of investigated medicinal products and are conducted in accordance with EU Directive 2001/20/ EMA - Note for Guidance on the Investigation of Bioavailability and Bioequivalence (CPMP/EWP/QWP/1401/98 Rev. 1/Corr**),

Objective: To evaluate and compare the bioavailability and therefore to assess the bioequivalence between a Test and Reference formulation of Azithromycin 500 mg when administered orally at the same dose level to 44 healthy volunteers under fasting conditions, in a 2-way crossover design.

To evaluate and compare the bioavailability and therefore to assess the bioequivalence between a

Material and methods: Clinical study included forty-four male healthy volunteers who met inclusion criteria and had signed Informed consent in accordance with the Clinical Study Protocol approved by Ethical Committee for clinical and other investigations related to medicines and medical devices at the Agency for Medicines and Medical Devices of Republic of North Macedonia. Validated HPLC-MS/MS method has been used for determination of Azithromycin in plasma, and with pharmacokinetic analysis, pharmacokinetics variables were determined: **Primary Variables**; AUC_{0-72h} **Secondary Variables**; AUC₀₋. **Statistical analysis:** An analysis of variance (ANOVA) was used to evaluate treatment, sequence and period effects. **Safety:** Descriptive statistics were calculated for demographic data and for adverse events.

Results: Pharmacokinetic: A total of 44 subjects had pharmacokinetic samples, 43 of them were included in the pharmacokinetic statistical analysis **Safety:** Both treatments (A and B) appear to be safe and well tolerated after single oral dose. No severe adverse events, serious adverse events or deaths

Conclusion: Both products are bioequivalent based on the results of main analysis (equivalence of AUC_{0-72h} and C_{max} using ln-transformed data).

Key words: Bioequivalence; Bioavailability; Azithromycin

Introduction

Azithromycin belongs to the macrolide group of antibacterials for systemic use. It is an azalide derived from erythromycin; however, it differs chemically from erythromycin in that a methyl-substituted nitrogen atom is incorporated into the lactone ring. Azithromycin acts by binding to the 23S rRNA of the 50S ribosomal subunit of susceptible microorganisms inhibiting bacterial protein synthesis and impeding the assembly of the 50S ribosomal subunit.

Azithromycin is indicated for the treatment of acute bacterial sinusitis, pharyngitis/tonsillitis, and otitis media, acute bacterial exacerbations of chronic bronchitis, community acquired pneumonia appropriate for oral therapy, uncomplicated skin and soft tissue infections, urethritis, cervicitis, genital ulcer disease, as well as other infections caused by susceptible microorganisms.

Azithromycin should not be used in patients with pneumonia who are judged to be inappropriate for oral therapy because of moderate to severe illness or risk factors. To reduce the development of drug-resistant bacteria and maintain the effectiveness of azithromycin and other antibacterial drugs, azithromycin

should be used only to treat infections that are proven or strongly suspected to be caused by susceptible bacteria.

After oral administration the bioavailability of azithromycin is approximately 37%. Peak plasma levels are reached after 2-3 hours (C_{max} after single dose of 500 mg azithromycin orally was approximately 0.4 mg/L). Kinetic studies have shown markedly higher azithromycin levels in tissue than in plasma (up to 50 times the maximum observed concentration in plasma) indicating that the active substance is heavily bound in tissue. The mean volume of distribution at steady state has been calculated to be 31.1 L/kg.

Concentration in target tissues such as lungs, tonsil, and prostate exceed the MIC_{90} for likely pathogens after a single dose of 500 mg. In experimental *in vitro* and *in vivo* studies azithromycin accumulates in the phagocytes, freeing is stimulated by active phagocytosis.

Plasma terminal elimination half-life closely reflects the elimination half-life from tissues of 2 to 4 days. Approximately 12% of an intravenously administered dose of azithromycin is excreted unchanged in urine within the following three days. Particularly high concentrations of unchanged azithromycin have been found in human bile. Also in bile, ten metabolites were detected, which were formed through N- and O- demethylation, hydroxylation of desosamine and aglycone rings and cleavage of cladinose conjugate. Comparison of the results of liquid chromatography and microbiological analyses has shown that the metabolites of azithromycin are not microbiologically active.

The most common treatment-related adverse reactions in adult patients receiving multiple-dose regimens of azithromycin were related to the gastrointestinal system with diarrhoea/loose stools, nausea, and abdominal pain being the most frequently reported. No other adverse reactions occurred in patients on the multiple-dose regimens of azithromycin with a frequency greater than 1%. Adverse reactions that occurred with a frequency of 1% or less included the following: palpitations, chest pain, dyspepsia, flatulence, vomiting, melena, cholestatic jaundice, monilia, vaginitis, nephritis, dizziness, headache, vertigo, somnolence, fatigue, rash, pruritus, photosensitivity, and angioedema.

Based on the EMA - *Note for Guidance on the Investigation of Bioavailability and Bioequivalence* (CPMP/EWP/QWP/1401/98 Rev. 1/Corr**), a single-dose comparative bioavailability study is adequate to demonstrate bioequivalence of an orally administered immediate-release formulation with systemic action.

Bioequivalence was tested under fasting conditions since this situation is usually more sensitive to show differences in pharmacokinetics. The evaluation of bioequivalence was based upon measured concentrations of the parent compound, which is in accordance with the bioequivalence guideline.

Study objective

The objective of this study was to compare the rate and extent of absorption of azithromycin from Test (Treatment A) versus Reference (Treatment B), as well as safety evaluation administered to healthy volunteers in a single-dose, randomized, 2 way cross-over study under fasting conditions, using a randomized two way crossover study in 44 healthy male volunteers after single oral dose under fasting conditions.

Material and methods

Forty-four male healthy volunteers (aged 19-49 years, with Ideal Body Weight according to the Body Mass Index 18-28, non-smokers) consisting of university students and members of the community at large, recruited from the Department of Pharmacology database of suitable clinical trial volunteers, were included in the study. All volunteers were medically checked up with health condition established based on history, physical examination, ECG, biochemical and hematological tests performed at the Institute of Preclinical and Clinical Pharmacology and Toxicology, prior to entering the study, all volunteers signed the Informed consent.

The following treatments were administered in the fasting state:

- Treatment T (Test): One Azimed 500 mg film coated tablet, administered with 240 ml of water after 10 hours fast.
- Treatment R (Reference): One Zithromax[®] 500 mg film coated tablet, with a 240 ml of water after 10 hours fast

The study was a single center, open, randomized, two - way crossover study in 24 healthy male volunteers after oral administration of single dose of investigated drug with a wash - out period of two weeks between administration of Test and Reference drug.

The volunteers received Test formulation according to the randomization scheme. After washout period of 14 days, Reference formulation was administered.

Results

Efficacy (pharmacokinetic), Safety Measurements Assessed and Flow chart

Azithromycin plasma concentrations achieved by administering a single dose of study medication (T or R) were measured using an LC/MS/MS method to determine the pharmacokinetic profile of the Test product in relation to the Reference product.

The main pharmacokinetic parameters for this study were C_{max} and AUC_{0-72h}.

C_{max}: Maximum observed plasma concentration, obtained directly from the data – without interpolation.

AUC_{0-72h}: Area under the plasma concentration time curve calculated from 0 to 72 hours, using the mixed model linear log trapezoidal rule. If there are concentrations below LLOQ (Lower Limit of Quantification) at 72 hours post-dose in at least one subject, then AUC_{0-t} was calculated for all volunteers instead of AUC_{0-72h}

Sample Collection

For determination of azithromycin in plasma a total of 20 blood samples were taken per period: at pre-dose and at 0.500, 1.000, 1.333, 1.667, 2.000, 2.250, 2.500, 2.750, 3.000, 3.500, 4.000, 5.000, 6.000, 8.000, 12.000, 16.000, 24.000, 48.000 and 72.000 hours post-dose (1 x 4 mL each).

The total volume of blood drawn from each subject completing the study did not exceed 160 mL, not including blood volume taken for clinical laboratory examination.

Samples were collected in vacutainers using direct venipuncture or an intravenous cannula in a forearm vein and processed as per sample processing instructions for azithromycin. Samples were maintained in ice/water bath from the start of collection to storage. The actual sampling times of all blood draws were recorded in the subject's sampling time sheet. Sampling time sheets for all subjects are included in the CRFs and are available on request.

Samples Handling

At each point, 4 mL blood samples were collected into blood collection tubes containing EDTA K₂ and centrifuged at 1900 g (± 38 g), at a temperature of + 4°C (± 4 °C) for 10 minutes. The maximum time between sample collection and start of centrifugation did not exceed 30 minutes. Obtained plasma was divided into polypropylene tubes in two aliquots. The recommended minimum volume of the first aliquot was 0.7 mL, and the recommended minimum volume of the second aliquot was 0.7 mL (or the remaining volume, if the plasma obtained is insufficient). All plasma samples were capped and stored frozen in an upright position within 30 minutes from end of centrifugation. Samples were stored in freezers at the clinical and bioanalytical facility at a temperature -20 ± 5 °C until assayed.

The tubes were labelled with the study code, number corresponding to the subject, study period, sampling time, analyte, analytical number and no. of the aliquot. The formulation identity was not revealed.

There were no deviations in sample processing.

Samples shipping

All aliquots of frozen samples were delivered on sufficient dry ice to keep the samples frozen for at least 72 hours using data logger to the bio analytical facilities: Anapharm Europe, S.L.U.; Encuny 22, 2nd floor, 08038, Barcelona, Spain.

The samples-to-analyse memos were prepared at the clinical site, confirmed by the sponsor and then sent to the designated bioanalytical facilities before the sample analysis start.

Medical Faculty of Ss. Cyril and Methodius University, Department of Preclinical and Clinical Pharmacology & Toxicology, Skopje, Republic of North Macedonia, was responsible for the organization of the transport. The transport was documented.

Analytical Treatment

Once in the bio analytical laboratory, test samples were stored at -20 ± 5 °C until performing measurement of Azithromycin concentrations.

The determination of azithromycin was performed by the bioanalytical division of Anapharm using the analytical method SOP ANE 5019 entitled “*Determination of Azithromycin in Human EDTA Plasma over a Concentration Range from 5 to 1000 ng/mL using an LC/MS/MS Method*”.

Details concerning the performance of the analytical method used during the analysis of the study samples and the final analytical results are given in the Analytical Reports presented separately.

Data Quality Assurance

Designated personnel for all clinical portions were responsible for maintaining quality assurance and quality control systems with written Standard Operating Procedures (SOPs) to ensure that the trial was conducted and data were generated and reported in compliance with the study protocol, GCP, GMP, GLP and other applicable regulatory requirements.

Pharmacokinetics:

Data from subjects who provide evaluable pharmacokinetic data for both Test and Reference products were included in the pharmacokinetic and statistical analysis for bioequivalence determination.

The pharmacokinetic parameters were estimated according to a non-compartmental approach with a log-linear terminal phase assumption using the drug concentration profiles and the actual sampling times with Phoenix® WinNonLin® v8.2.

Values below the lower limit of quantification in the absorption phase and in the terminal were set to zero.

AUC_{0-72h} (or AUC_{0-t}, if applicable) was calculated using the mixed model linear log trapezoidal rule.

The selection of the data points of the terminal log-linear phase was obtained automatically by WinNonLin® (using at least three available above LLOQ concentration points occurring after C_{max}).

The maximum plasma concentration (C_{max}) and the time of the peak concentration (t_{max}) were directly derived from the plasma concentration-time curve.

The level of significance was set to the standard value of 5% (0.05) for all statistical tests.

To determine bioequivalence, an analysis of variance (ANOVA) was performed for the estimated values (ln-transformed values) of C_{max} and AUC_{0-72h} (or C_{max}, AUC_{0-t} and AUC_{0-∞}, when applicable) using the SAS® System (Release 9.4 or an upgraded version).

The decision on bioequivalence was based on C_{max} and AUC_{0-72h} using ln-transformed data.

The following margins are pre-defined for the acceptance of bioequivalence:

- Ln [C_{max}] ± 20% range [80.00% - 125.00%]
- Ln [AUC_{0-72h}] ± 20% range [80.00% - 125.00%]

In case of not all subjects have quantifiable concentration levels at 72 hours post-dose, the decision on bioequivalence was to be based on C_{max} and AUC_{0-t} using ln-transformed data. The analysis of Ln [AUC_{0-∞}] was to be considered only as additional information. The following margins are pre-defined for the acceptance of bioequivalence:

- Ln [C_{max}] ± 20% range [80.00% - 125.00%]

- Ln [AUC_{0-t}] ± 20% range [80.00% - 125.00%]

The variability estimated from the residual error of the ANOVA model was to be used for the estimation of the 90% confidence interval.

Safety:

Descriptive statistics (mean, SD, min, max) were performed for subject’s demographics data (age, height, weight, BMI). The protocol asked for 44 healthy volunteers to be enrolled in this study.

The safety population was defined as all subjects who received at least 1 dose of the study medication.

For bioequivalence assessment, all ratios and 90% CI of main parameters, AUC_{0-72h} and C_{max} were determined.

The following safety assessments were done: assessment of past medical history, physical examination, measurement of vital signs at screening and before each dosing (body temperature, seated systolic and diastolic blood pressure and heart rate), ECG at screening, medical and drug therapy history and laboratory results (biochemistry, haematology and urinalysis), also tests for hepatitis B, C and HIV at screening. Subjects with positive results were not allowed to participate in the study.

Urine drug screen, alcohol test and cotinine test were performed at check-in of each period of the study. Other health events were recorded in the period between signing the informed consent form and the first study medication administration in Period 1.

Monitoring of adverse events was always done during the study. End-of-study clinical laboratory tests (biochemistry, haematology, urinalysis), end of study vital signs (blood pressure and heart rate) measurements were performed within suitable interval of time after end of the clinical part of the study (keeping in consideration the volunteer’s health conditions, safety aspects, validity of the results). Serum pregnancy tests for female subjects were performed at screening, check-in at each period and at the end of the study.

Efficacy (pharmacokinetic) results and tabulations of individual patient data

According to Statistical Analysis Plan (Final 01, 28.JUL.21), due to any subject showed concentrations below LLOQ (Lower Limit of Quantification) at 72 hours post-dose, AUC_{0-72h} is calculated for all volunteers. Moreover C_{max} and t_{max} are calculated for all subjects too.

All ratios and their 90% CI of main parameters, C_{max} and AUC_{0-72h}, laid within the pre-established margins of bioequivalence.

Table 1. Bioequivalence Assessment Summary

ln-transformed data*	Geometric LSMEANS		Bioequivalence assessment		Intra-subject coefficient of variation (%)**
			T/R Ratio (%)	90% CI [‡] (%)	
	Test	Reference			
C _{max} (ng/mL)	449.8436	424.1979	106.05	(97.50, 115.35)	23.47
AUC _{0-72h} (h*ng/mL)	3007.6069	2874.1148	104.65	(98.76, 110.89)	16.06

‡: classic parametric CI.

*: Analysis of variance according to the model of section **Error! Reference source not found.**

** : Intra-subject coefficient of variation= CV (%)= 100 x $\sqrt{e^{MSE} - 1}$. MSE=Mean Square Error.

Source: listings 1 and 2.

Ref.: Zithromax® 500 mg film-coated tablets (MAH: Laboratórios Pfizer, Lda.; Portugal, EU).

Test: Azithromycin 500 mg film-coated tablets (Azimed®) manufactured by Replek Farm LTD Skopje, Republic of North Macedonia

Adverse events

A total of 10 post-dose AEs were reported by 6 (13.64%) of the 44 subjects who received at least one dose of the study medication (safety population).

Three AEs (nausea-3) were reported by 6.98% (n=3) of the 43 subjects who received Treatment A. Seven AEs (headache-2, vomiting-2, anxiety-1, nausea-1, abdominal pain-1) were reported by 9.09% (n=4) of the 44 subjects who received Treatment B.

The most reported AE was nausea, reported by 9.09% (n=4) of subjects who constituted the safety population.

Of the 10 AEs reported, 8 were graded as mild and 2 were graded as moderate.

All AEs experienced during this study were resolved completely by the end of the study.

No severe adverse events, serious adverse events or deaths occurred during the study.

Upon conclusion of the clinical portion of the study, the results from the subjects who completed end of study procedures, including laboratory tests and vital signs measurement confirmed the absence of significant changes in the subject's state of health.

No new safety concerns related to administered formulations were raised during the conduct of this study.

Table 2. Summary of Adverse Events by System Organ Class

System Organ Class Term MedDRA Preferred Term	Reported Incidence by Treatment Groups	
	Bioequivalence Study	
	TREATMENT A (N = 43) n (%)	TREATMENT B (N = 44) n (%)
Subjects with one or more AE's	3 (6.98%)	4 (9.09%)
Gastrointestinal system disorders		
nausea	3 (6.98%)	1 (2.27%)
vomiting	/	2 (4.55%)
abdominal pain	/	1 (2.27%)
Nervous system disorders		
headache	/	2 (4.55%)
Psychiatric disorders		
anxiety	/	1 (2.27%)
<p>Although a subject may have had 2 or more AEs, the subject is counted only once within a treatment.</p> <p>The same subject may appear in different treatments.</p>		

Table 3. Overview of Adverse events

Parameter	Treatment A (N=43)	Treatment B (N=44)	Overall (N=44)
AEs reported	3	7	10
Subjects with at least one AE	3 (6.98%)	4 (9.09%)	6 (13.64%)
AEs relationship			
Probable	3 (100%)	3 (42.86%)	6 (60%)
Possible	0	2 (28.57%)	2 (20%)
Unlikely	0	2 (28.57%)	2 (20%)
Not related	0	0	0
Not possible to judge	0	0	0
AEs by Intensity			
Mild	3 (100%)	5 (71.43%)	8 (80%)
Moderate	0	2 (28.57%)	2 (20%)
Severe	0	0	0
SAEs reported	0	0	0
Deaths	0	0	0

Discussion and overall conclusion

No sequence, formulation or period effects were detected for the main pharmacokinetic parameters.

All ratios and their 90% CI of main parameters, AUC_{0-72h} and C_{max} laid within the pre-established margins of bioequivalence for the analysis according to the Study Protocol and SAP, i.e parametric approach with ln-transformed data.

Both products are bioequivalent based on the results of main analysis (equivalence of C_{max} and AUC_{0-72h} using ln-transformed data).

Upon conclusion of the clinical portion of the study, the results from the subjects who completed end of study procedures, including laboratory tests and vital signs measurement, confirmed the absence of significant changes in the subjects' state of health.

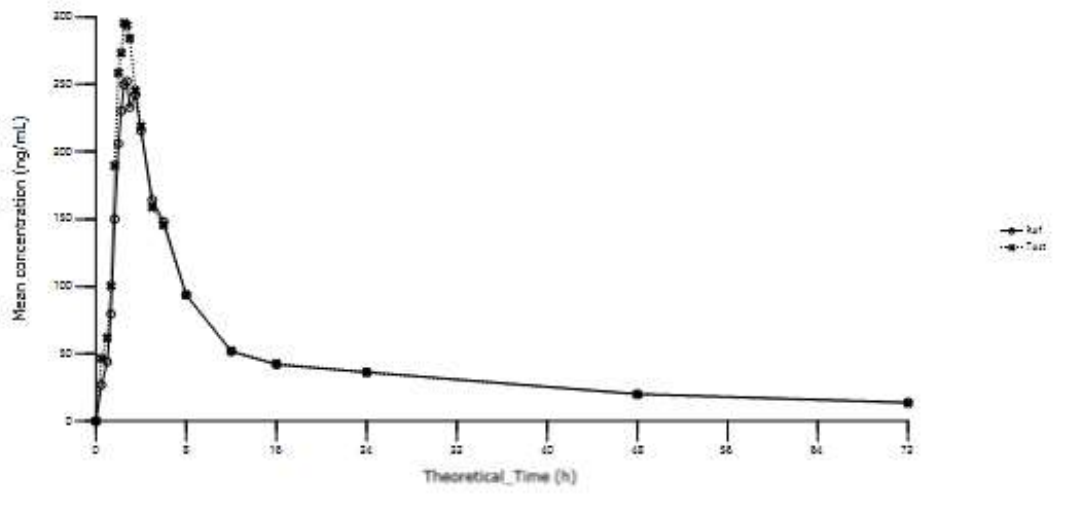
Both treatments (A and B) appear to be safe and well-tolerated after single oral dose of azithromycin 500 mg azithromycin 500 mg film coated tablets under fasting conditions in healthy male and female volunteers.

No serious or severe adverse events related to study treatments were observed during the study. No new safety concerns related to administered formulation were raised during the conduct of this study.

Table 4.

	Test [‡] (T)	Reference [‡] (R)	T/R Ratio [£] (%)	90% CI [£]
C _{max} (ng/mL) Mean (SD)	476.345 (160.481)	458.892 (174.341)	106.05	(97.50, 115.35)
AUC _{0-72h} (h*ng/mL) Mean (SD)	3111.255 (819.280)	3024.433 (962.977)	104.65	(98.76, 110.89)
t _{max} (h) Median (Min- Max)	2.50 (1.33-6.00)	2.50 (1.33-8.00)		

Study Code: 01_AZITHRO_2021/IZA-124-21



Graph 1. Mean concentration vs time curve of Azithromycin

References:

1. Committee for Proprietary Medicinal Products. (1996). *ICH Topic E3: Structure and content of clinical study reports* (CPMP/ICH/137/95). European Medicines Agency.
2. Committee for Proprietary Medicinal Products. (2001). *Directive 2001/20/EC of the European Parliament and of the Council of 4 April 2001 on the approximation of the laws, regulations and administrative provisions of the Member States relating to the implementation of good clinical practice in the conduct of clinical trials on medicinal products for human use*. Official Journal of the European Communities.
3. European Medicines Agency. (2010). *Guideline on the investigation of bioequivalence* (EMA/CPMP/EWP/QWP/1401/98 Rev. 1/Corr**). <https://www.ema.europa.eu>
4. European Medicines Agency. (2011). *Guideline on bioanalytical method validation* (EMA/CHMP/EWP/192217/2009 Rev.1 Corr.2). <https://www.ema.europa.eu>

5. European Medicines Agency. (2015). *Guideline on the pharmacokinetic and clinical evaluation of modified release dosage forms* (EMA/CHMP/EWP/280/96). <https://www.ema.europa.eu>
6. European Medicines Agency. (2017). *ICH guideline for good clinical practice E6 (R2)* (EMA/CHMP/ICH/135/95). <https://www.ema.europa.eu>
7. European Commission. (2010). *EudraLex: The rules governing medicinal products in the European Union, Volume 4: EU guidelines to good manufacturing practice—Annex 13: Investigational medicinal products* (ENTR/F/2/AM/an D(2010) 3374).
8. Medicines Evaluation Board. (n.d.). *Public assessment report: Azithromycine Orifarm 250 mg and 500 mg film-coated tablets* (EU procedure No. NL/H/1299/001-002/DC). <https://www.geneesmiddeleninformatiebank.nl>
9. Medicines and Healthcare products Regulatory Agency. (n.d.-a). *Public assessment report: Azithromycin 250 mg and 500 mg film-coated tablets (Strandhaven Ltd t/a Somex Pharma)*.
10. Medicines and Healthcare products Regulatory Agency. (n.d.-b). *Public assessment report: Azithromycin 250 mg and 500 mg film-coated tablets (Wockhardt UK Limited)*.
11. Organisation for Economic Co-operation and Development. (1997). *OECD principles on good laboratory practice*. OECD Publishing.
12. Pfizer Inc. (2019). *Zithromax (azithromycin): Full prescribing information*. <https://labeling.pfizer.com/showlabeling.aspx?id=511>
13. RxList. (2020). *Zithromax (azithromycin) drug information*. <https://www.rxlist.com/zithromax-drug.htm>
14. World Medical Association. (2013). *Declaration of Helsinki: Ethical principles for medical research involving human subjects*. <https://www.wma.net/policies-post/wma-declaration-of-helsinki-ethical-principles-for-medical-research-involving-human-subjects/>
15. Zhang, P. (2003). A simple formula for sample size calculation in equivalence studies. *Journal of Biopharmaceutical Statistics*, 13(3), 529–538. <https://doi.org/10.1081/BIP-120022772>