CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

213436Orig1s000

CLINICAL PHARMACOLOGY REVIEW(S)

Office of Clinical Pharmacology Review

NDA or BLA Number	NDA 213436
Link to EDR	\\CDSESUB1\evsprod\nda213436
Submission Date	11/06/20
Submission Type	Original NDA – 505(b)(2)
Brand Name	TRUDHESA
Generic Name	Dihydroergotamine Mesylate
Dosage Form and Strength	Solution for nasal spray (4 mg/ml)
Route of Administration	Nasal
Proposed Indication	Acute treatment of migraine with or without aura
Applicant	Impel NeuroPharma, Inc.
Associated IND	IND 130133
OCP Review Team	Xiaohan Cai, Ph.D., Gopichand Gottipati, Ph.D.

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(b) (4)

1. Executive Summary

Impel Pharmaceuticals submitted an original New Drug Application (NDA 213436), seeking approval for TRUDHESA (INP104) for acute treatment of migraine headaches with or without aura via 505(b)(2) regulatory pathway. The proposed product, INP104, is a single-use drug-device combination product, which includes a drug constituent – dihydroergotamine mesylate (DHE) (solution at 4 mg/mL) and a nasal spray device constituent. INP104 is intended to deliver DHE to upper nasal space. The two listed drugs (LDs) used in this application, *D.H.E. 45* intravenous (I.V.) injection[1] (NDA 005929) and *Migranal* nasal spray[2] (NDA 020148) were originally approved in the US in 1946 and1997 respectively.

This application relies on a single-dose pivotal relative bioavailability study IND104-101 conducted in healthy subjects and a long-term safety study INP104-301, evaluating the chronic intermittent use of INP104 in patients with acute migraine with or without aura. Briefly, IND104-101 was conducted in healthy subjects to demonstrate a pharmacokinetic (PK) bridge between the proposed product (TRUDHESA nasal spray 1.45 mg) and each of the two LDs, *D.H.E. 45*® IV injection (1 mg) and *Migranal*® nasal spray (2 mg) to enable reliance on FDA's previous findings for safety and efficacy respectively. The results from Study INP104-101 showed that the upper bound of the 90% confidence interval for the geometric mean ratios for AUC ($AUC_{0\text{-last}}$ and $AUC_{0\text{-inf}}$) and C_{max} of DHE between INP104 nasal spray and *D.H.E 45*® was ≤ 125%, suggesting that it is appropriate to bridge safety information from *D.H.E 45*®; and the lower bound of the 90% confidence interval for the geometric mean ratios for AUC ($AUC_{0\text{-last}}$ and $AUC_{0\text{-inf}}$) and C_{max} of DHE between INP104 nasal spray and *Migranal*® was ≥ 80%, suggesting that it is appropriate to bridge efficacy information from *Migranal*®. The relative bioavailability study conducted by the applicant provides an adequate scientific bridge (i.e., the exposures from INP104 is bracketed by those from the two LDs) for this 505(b)(2) application. Therefore, TRUDHESA can rely on *D.H.E. 45* and *Migranal* and borrow relevant information from their respective labels.

The applicant proposed to include a labeling statement about (b) (4) (b) (4) However, owing to uncertainties in the data quality and certain other limitations (b) (4) the review team does not agree with the applicant's proposal to include such information in TRUDHESA labeling.

The Office of Study Integrity and Surveillance (OSIS) was consulted for clinical and analytical site inspections for the pivotal relative bioavailability study IND104-101. The

[1] USPI of D.H.E. 45 I.V. injection: https://www.accessdata.fda.gov/drugsatfda_docs/label/2002/20148s7s8lbl.pdf

[2] USPI of Migranal nasal spray: https://www.accessdata.fda.gov/drugsatfda_docs/label/2019/020148Orig1s025lbl.pdf

OSIS noted that inspection was not warranted for these sites because they were inspected previously (DARRTS dated 1/26/2021).

2. Recommendations

The Office of Clinical Pharmacology (OCP) has reviewed the information submitted in the NDA and recommends approval based an adequate PK bridge demonstrated between INP104 nasal spray (1.45 mg) and listed drugs *D.H.E. 45* IV injection (1 mg) and *Migranal* nasal spray (2 mg) for the treatment of acute treatment of migraine headaches with or without aura in adults.

3. Background and Regulatory History

The applicant is seeking approval of TRUDHESA via 505(b)(2) pathway and intends to rely on FDA's findings of safety and efficacy of DHE based on results from the pivotal relative bioavailability study using *D.H.E. 45*® Injection (relied on for systemic safety) and *Migranal*® Nasal Spray (relied on for efficacy) as the listed drugs (LDs).

In 2017, the FDA provided Written Responses to the Type C Meeting with the applicant's questions on the clinical development program. In the pre-NDA meeting (dated June, 2020), the applicant discussed with the agency about the proposed 505(b)(2) regulatory drug development program, including a relative BA study of INP104-101 and a long term Phase 3 study INP104-301, and the appropriateness of PK bridging strategy for INP104 NDA submission.

4. Summary of Pivotal Relative Bioavailability Study

Title: A Phase 1, Three-Period, Three-Way, Randomized, Open-Label, Single-Dose, Cross-Over, Comparative Bioavailability Study of Dihydroergotamine Mesylate (DHE) Administered by I123 Precision Olfactory Delivery (POD®) Device Nasal Spray, DHE for Injection (Intravenous), and Migranal® Nasal Spray in Healthy Adult Subjects

Primary Objectives:

- To compare the BA of DHE following a single dose administration by INP104 to that of D.H.E. 45 and Migranal in healthy adult subjects

Study Design and Methodology:

The study was an open-label, randomized, single-dose, three-period, six-sequence, three-way crossover, trial conducted in healthy adult subjects. A total of 38 subjects were enrolled to receive the 1 of 6 sequences (N = 6 per sequence). Each sequence contained 3 dosing periods involving administration of 1 of the following treatments:

	Treatment A	**Treatment B**	**Treatment C**
Product Administered	INP104	D.H.E. 45	Migranal
Dose/Strength	1.45 mg [4 mg/ml]	1 mg [1 mg/ml]	2 mg [4 mg/ml]
Route of Administration	Intranasal	Intravenous injection	Intranasal
Other Characteristics	self-administered through 2 actuations, 1 to each nostril	IV injection over 1 minute	self-administered as one spray in each nostril, followed by another single spray into each nostril 15 minutes later

The washout period was 7 days between the dosing days of any two consecutive periods. To mitigate the risk of nausea and vomiting associated with the use of D.H.E. 45, all subjects received 10 mg metoclopramide by slow IV injection over 1 to 3 minutes within 5 to 10 minutes prior to administration of all three investigational products.

Number of Subjects (Planned and Analyzed)

A total of 38 subjects enrolled in the study, 36 subjects received at least one dose of investigational product (IP). A total of 29 subjects received all three scheduled doses of IP.

Post-dose nasal leakage following the administration of Migranal and INP104 were self-reported in 76.5% (N=26) and 32.3% (N=10) of subjects, respectively. The clinical staff also observed and reported leakage of IP following drug administration in at least one nostril in 58.8% (N=20) and 16.1% (N=5) of subjects dosed with Migranal and INP104, respectively.

PK Sampling:

PK blood samples were collected at pre-dose, post-dost at 5, 10, 20, 30, 40 and 50 minutes, and 1, 1.25, 1.5 1.75, 2, 3, 4, 8, 12, 24, 36 and 48 hours. Plasma concentration of DHE and 8'-OH-DHE was quantified using a validated LC-MS/MS assay (refer to section 5).

Criteria for Evaluation

Pharmacokinetic parameters were computed from the individual plasma DHE and 8'-OHDHE concentrations using a non-compartmental approach. Only subjects with data values for all of the variables for each of the 3 doses were to form the primary PK Population (used for comparative BA assessment).

For the primary comparative bioavailability assessment, the following criteria must be met to conclude that this study successfully establishes the scientific bridge to the DHE for Injection (IV) and Migranal Nasal Spray products.

1. The upper bound of the 90% confidence intervals of the INP104 to DHE for Injection (IV) geometric mean ratios for DHE AUC_{0-t}, AUC_{0-inf} and C_{max} are ≤ 125%, and

2. The lower bound of the 90% confidence intervals of the INP104 to Migranal Nasal Spray geometric mean ratios for DHE AUC_{0-t}, AUC_{0-inf} and C_{max} are ≥ 80%.

Pharmacokinetics Results:

<u>DHE</u>

Based on the applicant's PK analysis, 27 subjects were included in the primary PK population and two subjects ((b) (6) and (b) (6) were excluded from the PK

population because the exposure metrics could not be computed. Specifically, the sponsor's analysis concluded that subjects (b) (6) and (b) (6) had insufficient data (for D.H.E. 45 and Migranal, respectively) for calculation of either C_{max}, $AUC_{0\text{-}last}$ or $AUC_{0\text{-}inf}$. The PK and BA analysis conducted by the applicant are presented as below:

Table 1: Study INP104-101: Geometric Mean Ratios (90% CI) of DHE following a Single Dose of INP104 and Migranal (Applicant's Analysis)

	INP104 (1.45 mg DHE)		Migranal (2 mg DHE)		Ratio of Geometric Means (%) (INP104/Migranal)	One-sided 90% CI for Ratio of Geometric Means (%)	
Pharmacokinetic Parameter (Units)	**n**	**Geometric Mean**	**n**	**Geometric Mean**		**Lower**	**Upper**
$AUC_{0\text{-}inf}$ (h*pg/mL)	27	5562	27	1809	307.5	**235.7**	401.2
$AUC_{0\text{-}last}$ (h*pg/mL)	27	5194	27	1576	329.6	**245.6**	442.2
C_{max} (pg/mL)	27	1131	27	254.0	445.1	**329.3**	601.6

Source: Tables 13 and 16 of Study Report INP104-101

Table 2: Study INP104-101: Geometric Mean Ratios (90% CI) of DHE following a Single Dose of INP104 and D.H.E. 45 (IV) (Applicant's Analysis)

	INP104 (1.45 mg DHE)		D.H.E. 45 (1 mg DHE)		Ratio of Geometric Means (%) (INP104/D.H.E. 45)	One-sided 90% CI for Ratio of Geometric Means (%)	
Pharmacokinetic Parameter (Units)	**n**	**Geometric Mean**	**n**	**Geometric Mean**		**Lower**	**Upper**
$AUC_{0\text{-}inf}$ (h*pg/mL)	27	5485.	27	7392	74.20	60.34	**91.24**
$AUC_{0\text{-}last}$ (h*pg/mL)	27	5119.	27	6978	73.36	58.91	**91.35**
C_{max} (pg/mL)	27	1115	27	14140	7.883	6.198	**10.03**

Source: Tables 13 and 16 of Study Report INP104-101

Reviewer's Comments: The reviewer has comments on the PK population, exclusion of certain concentration data, and post-dose nasal leakage issue on the nasal spray products:

1. *For PK population, the reviewer does not agree with excluding subjects* (b) (6) *and* (b) (6) *from the PK population for the following reasons:*
   - *Subject* (b) (6)*: Sufficient data were available to compare the* C_{max} *and* $AUC_{0\text{-}last}$ *between the treatments of INP-104 and Migranal. The* $AUC_{0\text{-}inf}$ *after receiving Migranal should be excluded for comparison based on the protocol requirement because the adjusted* R^2 *was below 0.80. The* C_{max}*,* $AUC_{0\text{-}last}$ *or* $AUC_{0\text{-}inf}$ *for treatment of D.H.E 45 cannot be estimated due to issues with sample collection at 5 min, 10 min, 20 min, and 30 min.*
   - *Subject* (b) (6)*: Sufficient data were available for the comparison of the* C_{max}*,* $AUC_{0\text{-}last}$ *, and* $AUC_{0\text{-}inf}$ *between the treatments of INP-104 and D.H.E 45. For the PK comparison between the treatment of INP-104 and Migranal, there were sufficient data to compare the* C_{max} *and* $AUC_{0\text{-}last}$*, but not for* $AUC_{0\text{-}inf}$*, because the* λ *of the Migranal treatment cannot be estimated.*
2. *Further, although subject* (b) (6) *did not receive the treatment of D.H.E 45 and did not complete the study, there were sufficient data allowing PK analysis from treatments of INP104 and Migranal. Therefore, subject* (b) (6) *was included in the reviewer's analysis.*
3. *In addition, the reviewer does not agree with including the PK data from subject* (b) (6) *for the treatment of D.H.E 45 because subject* (b) (6) *received partial dose (approximately 90%) during the drug administration. For subject* (b) (6) *PK data from the INP104 and Migranal treatments were included in the reviewer's PK and BE analysis.*

Table 3: Study INP104-101: Summary of Plasma PK Parameters of DHE (PK Population, *Reviewer's Analysis*)

	Treatment					
	1.45 mg INP104		1 mg D.H.E. 45 for injection (IV)		2 mg Migranal	
Parameters	**N**	**Mean (CV%)**	**N**	**Mean (CV%)**	**N**	**Mean (CV%)**
T_{max} (h)[#]	30	0.5 (0.33-2.05)	27	0.08 (0.07-0.1)	30	0.73 (0.5-3.08)
C_{max} (pg/mL)	30	1277 (52.2%)	27	14491.48 (34.4%)	30	329.26 (84.9%)
$AUC_{0\text{-last}}$ (h*pg/mL)	30	5951.89 (42.8%)	27	8424.03 (56.1%)	30	2057.2 (79.7%)
$AUC_{0\text{-inf}}$ (h*pg/mL)	30	6247.04 (42.1%)	27	8798.72 (53.7%)	28	2381.15 (70.6%)
$t_{½}$ (h)	30	11.69 (24.2%)	27	14.52	28	10.73 (27.9%)

[#]T_{max} results are expressed as Median (Minimum – Maximum)

*N=28 for elimination phase dependent pharmacokinetic parameters of Migranal 2 mg because subjects (b) (6) and (b) (6) had R2 adjusted < 0.80

4. *The reviewer does not agree with the applicant's decision to exclude certain concentration data without assignable causes. Based on the time-concentration profile, the applicant excluded the following concentration data from three subjects receiving D.H.E. 45 treatment because the concentration had >10SD from the mean and they appeared to be inconsistent with the expected concentration-time profile (Refer to Figure 1.1 and 1.2).*

   - *Subject (b) (6) 40 min post dose of D.H.E. 45 treatment*
   - *Subject (b) (6): 8 hr post dose of D.H.E. 45 treatment*
   - *Subject (b) (6) 8 hr post dose of D.H.E. 45 treatment*

However, based on the clinical report and analytical report of Study INP-104-101, there is no assignable causes for excluding the above concentration data. Therefore, the reviewer's analysis included these three concentrations.

Figure 1.1: Study INP104-101: Mean Plasma Concentration vs. Time Curve for DHE (Reviewer's Analysis, PK Population with outlier concentration data excluded)

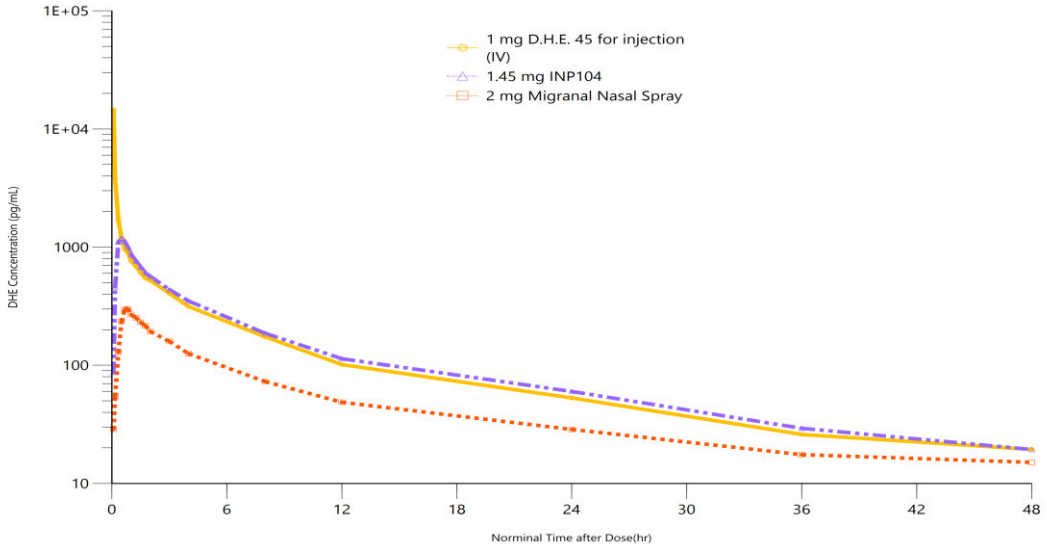

Figure 1.2: Study INP104-101: Mean Plasma Concentration vs. Time Curve for DHE (Reviewer's Analysis, PK Population- without concentration data exclusion)

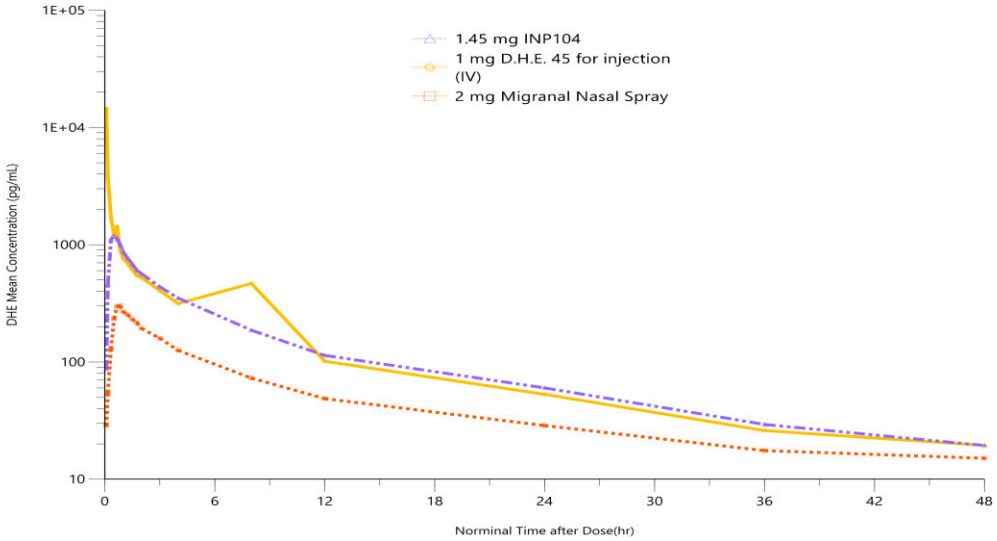

Table 4: Study INP104-101: Geometric Mean Ratios (90% CI) of DHE following a Single Dose of INP104 and D.H.E. 45 (IV) (Reviewer's Analysis)

	Geometric Least Square Means			90% Confidence Interval	
Parameters	**INP104 1.45 mg (N=30)**	**1 mg D.H.E. 45 for injection (IV) (N=27)**	**Ratio% (INP104/D.H.E. 45)**	**Lower**	**Upper**
C_{max} (pg/mL)	1108	14024	7.9	5.96	**10.47**
$AUC_{0\text{-}last}$ (h*pg/mL)	5267	7891	66.7	50.01	**89.08**
$AUC_{0\text{-}inf}$ (h*pg/mL)	5582	8263	67.6	52.62	**86.73**

Table 5: Study INP104-101: Geometric Mean Ratios (90% CI) of DHE following a Single Dose of INP104 and Migranal (Reviewer's Analysis)

	Geometric Least Square Means			90% Confidence Interval	
Parameters	**INP104 1.45 mg (N=30)**	**Migranal 2 mg Nasal Spray (N=30)**	**Ratio (INP104/Migranal)**	**Lower**	**Upper**
C_{max} (pg/mL)	1108	232	478.4	**363.93**	628.86
$AUC_{0\text{-}last}$ (h*pg/mL)	5267	1485	354.8	**268.02**	469.63
$AUC_{0\text{-}inf}$ (h*pg/mL)	5582	*1893	294.8	**230.15**	377.71

*N=28

5. *Post-dose nasal leakage issue was reported after the administration of Migranal and INP-104 nasal spray products. The bioavailability (BA) of Migranal nasal spray was determined as 11.5% in Study INP-104-101, as the geometric mean ratio of the $AUC_{0\text{-}inf}$ between Migranal and D.H.E 45 IV treatments based on the reviewer's analysis. The higher percentage of subjects experiencing dripping from nose in after Migranal treatment (76.5%) may contribute to high inter-subject variability in PK exposure,*

when comparing to those receiving INP104 (32.3%). However, the issue of post-dose nasal leakage is not expected to have significant impact on the study conclusion for the following reasons:

- *Based on the published literature on conventional nasal spray products used for migraine treatment, spillage of solutions and liquid medication dripping out of the nose after administration were reported[3][4]. The inconsistent drug delivery may contribute different nasal absorption.*
- *The USPI for Migranal indicates an absolute BA of 32%, while in the current study INP-104-101, the absolute BA was found to be 11.5% (i.e., 2.8-fold lower). Based on the results indicated in Table-5, the GMR (point estimate) for all the DHE exposure metrics exceeds the 2.8-fold difference noted between INP104 and Migranal treatments. These results indicate that GMR (point estimate) of DHE exposures provides adequate coverage for differences in the absolute BA between Migranal labeling and INP-104-101 noted above, suggesting that differences in post-dose nasal leakage between Migranal and INP104 are unlikely to affect the study conclusions.*

Discussion and Conclusion

The comparative bioavailability of INP104 to Migranal and D.H.E 45 was based on the DHE PK data following a single dose of INP104 at 1.45 mg, Migranal at 2 mg, and D.H.E 45 at 1 mg. When comparing the DHE PK between INP104 and the reference product D.H.E. 45, the upper bound of the 90% CI for the ratio of the geometric means for DHE AUC ($AUC_{0\text{-last}}$ and $AUC_{0\text{-inf}}$) and C_{max} was ≤ 125%. When comparing the DHE PK between INP104 and the reference product Migranal, the lower bound of the 90% CI for the ratio of the geometric means for DHE AUC ($AUC_{0\text{-last}}$ and $AUC_{0\text{-inf}}$) and C_{max} was ≥ 80%. Median T_{max} of DHE following the treatment of INP104 (0.5 hr) was found to be within the T_{max} range of D.H.E. 45 and Migranal (0.73 hr).

The proposed dosing regimen for INP104 for the acute treatment of migraine headaches with or without aura is a single dose of 1. 45 mg DHE, as one spray into each nostril. The maximum dose was 2 doses within a 24-hour period and 3 doses within a 7-day period. The proposed dosing recommendation as a single dose was based on the comparative BA results from Study INP104-101. The information supporting maximum recommended

[3] Silberstein SD, Shrewsbury SB, Hoekman J. Dihydroergotamine (DHE) - Then and Now: A Narrative Review. *Headache*. 2020;60(1):40-57. doi:10.1111/head.13700

[4] Tepper SJ. Clinical implications for breath-powered powder sumatriptan intranasal treatment. Headache. 2013;53(8):1341-1349. doi:10.1111/head.12166

doses of INP104 included the established PK bridge between INP104 and LDs, and PK extrapolation:

- Based on the established efficacy bridge established between INP104 at 1.45 mg and Migranal at 2 mg, the DHE exposure following two doses of INP104 (a total of 2.9 mg DHE) is expected to be greater than those following one and half doses of Migranal (a total of 3 mg DHE) administered within a 24-hour period. The DHE exposure following three doses of INP104 (a total of 4.35 mg DHE) is expected to be greater than those following two doses of Migranal (a total of 4 mg DHE) administered within a 7-day period. For Migranal, the maximum recommended dose is 3 mg within 24 hours and is 4 mg in a 7-day period.
- Based on the established safety bridge established between INP104 at 1.45 mg and D.H.E 45 at 1 mg, the DHE exposure following two doses of INP104 (a total of 2.9 mg DHE) is expected to be lower than those following two doses of D.H.E 45 (a total of 2 mg DHE) administered within a 24-hour period. The DHE exposure following three doses of INP104 (a total of 4.35 mg DHE) is expected to be lower than those following six doses of D.H.E 45 (a total of 6 mg DHE) administered within a 7-day period. For D.H.E 45, the maximum recommended dose is 2 mg within 24 hours and 6 mg in a 7-day period.

Additionally, the applicant conducted a Phase 3 Study INP104-301 to evaluate the long-term safety of INP104. Refer to the clinical review on the evaluation on INP104 safety information from Study INP104-301.

No DDI studies have been conducted with INP104. The applicant relied on DDI findings of INP104 from the approved labeling of the LDs. The applicant also proposed to (b) (4)

(b) (4)

(b) (4)

However, due to the uncertainties on the data quality and limitations, including insufficient information (b) (4)

the review team does not

(b) (4)

agree with including the [redacted] (b) (4) in the INP104 labeling (Refer to section 6.1).

5. Summary of Bioanalytical Method Validation and Performance

Plasma concentrations of DHE were measured by a validated LC-MS/MS method in human plasma for study INP104-101. Plasma samples from study INP104-101 were analyzed by [redacted] (b) (4) Because approval of INP104 relies on the pivotal relative BA study INP104-101, a routine inspection of the clinical and bioanalytical sites was requested via Office of Study Integrity and Surveillance (OSIS). Inspections are not warranted at this time for the clinical site, Nucleus Network, Ltd, Victoria, Australia or at the analytical site, [redacted] (b) (4) because of the previous inspection history in December 2017 and [redacted] (b) (4), respectively (Refer to NDA 213436, Bioequivalence Establishment Inspection Report Review, DARRTS, 1/26/2021).

Reviewer's comments: Method validation and sample analysis were acceptable.

Description of method validation parameters for DHE (validation study 0068-1886) are provided below:

Table 1: Bioanalytical Method Validation Summary

Parameter	Results	
Experimental start date	10-Nov-2017 [d]	
Experimental completion date	14-Nov-2017	
Methodology	Supported liquid extraction and LC-MS/MS instrumental analysis	
Species, Matrix	K_2EDTA human plasma	
Analytes to be measured	**Dihydroergotamine**	**8'-Hydroxy Dihydroergotamine**
Range for each analyte	10.0 to 10,000 pg/mL	20.0 to 20,000 pg/mL
Assay Sample Volume	200 µL	
Regression	Linear, $1/x^2$ weighting, 8 calibration standards in duplicate	
Internal Standard	Dihydroergotamine-IS	8-OH-DHE-IS
Inter-run % Bias	LLOQ VS (10.0 pg/mL): -3.4% Low VS (30.0 pg/mL): -2.3% Middle VS (750 pg/mL): -0.5% High VS (7500 pg/mL): 1.5%	LLOQ VS (20.0 pg/mL): 2.0% Low VS (60.0 pg/mL): -2.2% Middle VS (1500 pg/mL): -2.7% High VS (15,000 pg/mL): -1.3%
Inter-run % CV	LLOQ VS (10.0 pg/mL): 5.5% Low VS (30.0 pg/mL): 6.9% Middle VS (750 pg/mL): 1.6% High VS (7500 pg/mL): 2.3%	LLOQ VS (20.0 pg/mL): 11.2% Low VS (60.0 pg/mL): 3.8% Middle VS (1500 pg/mL): 3% High VS (15,000 pg/mL): 4.7%
Dilution Integrity	Over-range QC: 20,000 pg/mL diluted 10-fold Mean bias: -3%, Precision: 2.2%	Over-range QC: 40,000 pg/mL diluted 10-fold Mean bias: -2%, Precision: 2%
Carryover	≤ 6.2% for Dihydroergotamine, and ≤ 6.3% for 8'-Hydroxy Dihydroergotamine	
Selectivity	No interferences in 10 lots of matrix, including 2 hemolyzed and 2 lipemic lots	

Parameter	Results	
Spiked Selectivity	For 10 lots spiked to contain 30.0 pg/mL: Mean bias: -3.3%, Precision: 2.6%	For 10 lots spiked to contain 60.0 pg/mL: Mean bias: -3.5%, Precision: 3.1%
Hemolyzed Samples	Acceptable precision and accuracy (less than 15% CV and mean bias)	
Lipemic Samples	Acceptable precision and accuracy (less than 15% CV and mean bias)	
Analytes to be measured	**Dihydroergotamine**	**8'-Hydroxy Dihydroergotamine**
Recovery	78.8-87.1% for Dihydroergotamine and 84.0-94.6% for Dihydroergotamine-IS	68.1-82.9% for 8'-Hydroxy Dihydroergotamine and 71.8-90.5% for 8-OH-DHE-IS
Matrix Effects (ISTD normalized matrix factor)	Low level: 1.010, 4.6% CV High level: 0.999, 2.6% CV	Low level: 0.958, 8.8% CV High level: 1.050, 4.1% CV
Benchtop Stability	At least 23.5 hours at room temperature	
Freeze/Thaw Stability	At least 4 cycles, at either -20°C or -80°C	
Long Term Stability	At least 137 days, at either -20°C or -80°C	
Extract Stability	At least 66 hours for samples maintained at 2-8°C until injection	
Whole Blood Stability	At least 2 hours at room temperature, over multiple points	
Stock Solution Stability	At least 80 days at 0.200 mg/mL in ethanol when stored at -30°C to -10°C	At least 88 days at 0.200 mg/mL in ethanol when stored at -30°C to -10°C
Interference Assessments	Acceptable for both analytes	
Long Run Lengths	Acceptable for up to two full plates	

Source: Method validation report of study 0068-1886, page 9-10

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