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By Electronic Submission

Division of Dockets Management
Food and Drug Administration
Department of Health and Human Services
5630 Fishers Lane, Room 1061
Rockville, MD 20852

CITIZEN PETITION

On the behalf of our client, Apicore Pharmaceutical Private, Ltd. ("Apicore"), the undersigned ("Petitioner") submits this Citizen Petition under the Federal Food, Drug, and Cosmetic Act (FDCA), and 21 C.F.R. § 10.30, to request the Commissioner of Food and Drugs to require that any Abbreviated New Drug Application (ANDA) (or supplement to an ANDA) for Penicillamine capsules be approved only if the bioequivalence study supporting such approval uses a bioanalytical method that stabilizes Penicillamine from oxidation as soon as the blood samples are collected and measures copper-bound Penicillamine, along with the circulating free Penicillamine, that appears in plasma. ANDA methods that do not account for stabilization nor the release of copper-bound Penicillamine present a risk that the proposed product is not bioequivalent, and hence does not have the therapeutic benefit described in the labeled claims.

I. Action Requested

Petitioner requests the Commissioner of Food and Drugs to require that any ANDA (or supplement to an ANDA) for Penicillamine capsules be approved only if the bioequivalence study supporting such approval uses a bioanalytical method that introduces a stabilizing agent to minimize the degradation of free Penicillamine post sample collection and includes steps to measure Penicillamine from Penicillamine-copper complex.

II. Statement of Grounds

The bioanalytical method used by Apicore in ANDA No. 210646 Penicillamine Capsules USP (250 mg) to estimate the amount of therapeutic Penicillamine overcomes significant drawbacks of the existing bioanalytical methods, and should be used by pending and subsequent applications for Penicillamine capsules. Specifically, the Apicore method estimates the true concentration of Penicillamine that is present at the time of blood sample collection by (i) introducing a stabilizing agent that minimizes the oxidation of Penicillamine after sample

collection, and (ii) measuring free penicillamine from Penicillamine-copper complex, along with the circulating free Penicillamine (not bound to copper). Current methods do not account for stabilization, nor the release of copper-bound Penicillamine, and therefore present a risk that the proposed ANDA product is not bioequivalent, and hence does not have the therapeutic benefit described in the labeled claims.

A. An Analytical Method That Introduces a Stabilizing Agent Slows Oxidation of Penicillamine, Which Provides a More Accurate Measure of Free Penicillamine in the Bloodstream

In bioequivalence PK (pharmacokinetic) studies, Penicillamine is measured in a patient's bloodstream after oral administration. Immediately after patient blood sample collection, however, Penicillamine degrades into disulfide conjugates, including Penicillamine-disulfide and cysteine-Penicillamine disulfide, due to the oxidation of Penicillamine.[1]

The oxidation that occurs after sample collection is non-reversible. To measure accurately the amount of Penicillamine at the time of blood collection, an analytical method therefore needs to account for the oxidation of Penicillamine (and arrest the degradation of Penicillamine into Penicillamine-disulfides).

Adding a stabilizer at the time of blood sample collection minimizes oxidation of Penicillamine in the patient blood sample. Adding a stabilizing agent, which arrests oxidation, to collection vacutainers immediately upon blood sample collection preserves the free Penicillamine. Failure to add a stabilizing agent leads to the loss of Penicillamine in favor of Penicillamine-disulfide. The formation of Penicillamine-disulfide requires two molecules of Penicillamine, which takes away free Penicillamine from the blood concentration, thereby exaggerating the loss of Penicillamine and underestimating the amount of Penicillamine in the bloodstream at collection.

The stability of Penicillamine in plasma was evaluated under the conditions identified in **Figure 1**. **Figure 1** shows the stability of Penicillamine in plasma, with and without a stabilizing agent. Ethylenediaminetetraacetic acid (EDTA) also was present in the sample collection vacutainer. (EDTA functions by binding calcium in the blood, which keeps the blood from clotting.) The graph shows that the addition of a stabilizing agent slows the rate of oxidation and hence loss of free Penicillamine. Under all treatment conditions, some degradation was noted, however the addition of stabilizing agent demonstrated the slowest rate of diminution.

[1] See, e.g., Netter P. et. al., "Clinical Pharmacokinetics of Penicillamine-D," Clin. Pharmacokinetics 13, 317-333 (1987) (Attachment 1); Webb et. al., "Identification of factors limiting the accurate measurement of plasma D-penicillamine in rheumatoid arthritis patients," Ann Clin Biochem 25: 186-191 (1988) (Attachment 2).

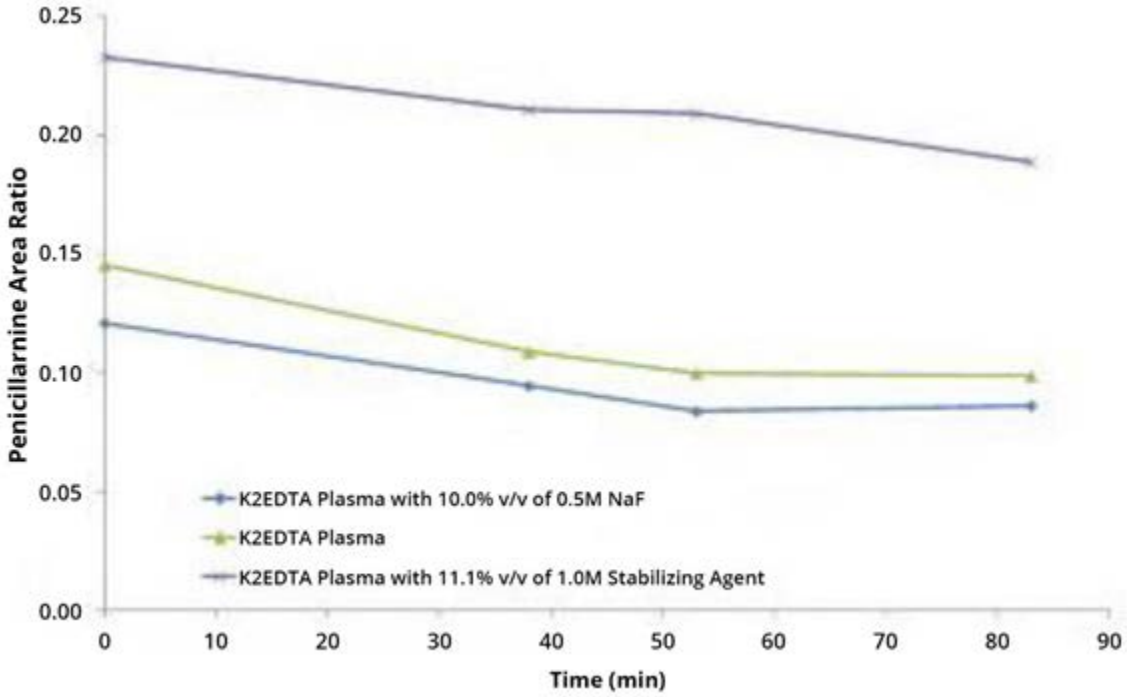

Figure 1. Kinetic in Plasma - Results with Apicore Extraction Method

In contrast, **Table 1** shows data from use of the bioanalytical method in the New Drug Application (NDA) CUPRIMINE® Penicillamine capsules (NDA 019853), the reference listed drug (RLD). The method, the trichloroacetic acid (TCA) crash method, suggests that there is significant loss of Penicillamine when the sample is processed only after 30 minutes of collection. After 30 minutes, loss of free Penicillamine ranges from 31% to 92%. The conversion from Penicillamine to Penicillamine-disulfide happens *in vitro*, after sample collection.

Extraction method	Condition	Donor	Average Area Ratio	% of Fresh Penicillamine QC Donor 1
TCA Crash	Freshly Treated (Treated with TCA within 2 min)	1	2.0427	N/NP
		2	1.8089	88.6%
		3	1.9439	95.2%
	(Treated with TCA within 30 min)	1	1.4052	68.8%
		2	0.1683	8.2 %
		3	0.5620	27.5%

Table 1: Donor comparison with TCA crashed penicillamine in plasma (at t=2 min and t=30 min after fortification)

The method also shows high variability among donors of Penicillamine detection, and is subjective to the analyst handling the sample preparation. (Results can vary significantly between analysts.)

Table 2 shows data from LC/MS/MS (liquid chromatography/ mass spectrometry) using the TCA crash method. The data shows that, without a stabilizing agent, a sample that is processed within 30 minutes of collection, shows loss of Penicillamine ranging between 37% and almost 97%. The TCA crash method, therefore, does not estimate accurately the free concentration of Penicillamine present in blood at the time of sample collection because significant degradation of the therapeutic concentration is seen post collection.

By adding a stabilizing agent to minimize the oxidation of Penicillamine into Penicillamine-disulfides, the method can more accurately estimate the amount of free penicillamine at the time of sample collection.

Short-Term Conditions	Matrix Type	Sample	Concentration (µM)	Equivalent Penicillamine Concentration (µM)	Matrix Lot	Average Area Ratio			% of Comparison			% of Spiked Pencillamine Accounted For
						Monitoring Penicillamine	Monitoring Penicillamine-Penicillamine Disulfide	Monitoring Penicillamine-Cysteine Disulfide	Monitoring Penicillamine	Monitoring Penicillamine-Penicillamine Disulfide	Monitoring Penicillamine-Cysteine Disulfide	
24h at 22°C	Plasma (Without Stabilizing Agent)	High QC Penicillamine	40.2	40.2	1	0.03420	2.13887	16.18543	1.7%	12.2%	40.1%	66.3%
					2	0.01475	6.95367	5.05703	0.7%	38.5%	13.5%	91.3%
					3	0.01546	4.14954	11.83628	0.8%	24.6%	30.5%	80.4%
		High QC Penicillamine-Penicillamine Disulfide	20.1	40.2	1	0.01492	18.04654	0.36448	0.7%	103.3%	0.9%	104.1%
					2	0.01108	17.54563	0.51028	0.6%	97.2%	1.4%	98.2%
					3	0.00952	17.86754	0.46525	0.5%	105.8%	1.2%	106.6%
		High QC Pencillamine-Cysteine Disulfide	40.2	40.2	1	0.03513	0.20341	30.59826	1.7%	1.2%	75.9%	79.9%
					2	0.02814	0.67387	28.67406	1.4%	3.7%	76.3%	85.2%
					3	0.02610	0.27984	28.09233	1.3%	1.7%	72.5%	77.0%
	TCA Crash Supernatant (Without Stabilizing Agent)	High QC Penicillamine	40.2	40.2	1	1.92838	0.08507	0.09801	94.4%	0.5%	0.2%	95.6%
					2	1.75571	0.79697	0.51590	88.0%	4.4%	1.4%	98.2%
					3	1.90361	0.28324	0.50244	93.2%	1.7%	1.3%	97.8%
		High QC Penicillamine-Penicillamine Disulfide	20.1	40.2	1	0.02958	17.35118	0.01935	1.4%	99.3%	0.0%	100.1%
					2	0.02581	17.87389	0.03721	1.3%	99.1%	0.1%	99.8%
					3	0.02002	16.67627	0.03519	1.0%	98.7%	0.1%	99.3%
		High QC Pencillamine-Cysteine Disulfide	40.2	40.2	1	0.01994	0.18854	39.19443	1.0%	1.1%	97.2%	100.3%
					2	0.01933	0.17143	38.34662	1.0%	1.0%	102.1%	104.9%
					3	0.01470	0.18722	39.64656	0.7%	1.1%	102.2%	105.2%
24h at 4°C	Plasma (Without Stabilizing Agent)	High QC Penicillamine	40.2	40.2	1	0.04211	2.92315	14.51473	2.1%	16.7%	36.0%	71.5%
					2	0.01796	6.86090	5.51636	0.9%	38.0%	14.7%	91.6%
					3	0.02009	4.11862	13.06436	1.0%	24.4%	33.7%	83.4%
		High QC Penicillamine-Penicillamine Disulfide	20.1	40.2	1	0.01738	18.44299	0.24392	0.9%	105.6%	0.6%	106.3%
					2	0.00609	17.57351	0.51263	0.3%	97.4%	1.4%	98.2%
					3	0.00746	17.02408	0.44080	0.4%	100.8%	1.1%	101.5%
		High QC Pencillamine-Cysteine Disulfide	40.2	40.2	1	0.03254	0.18257	37.20996	1.6%	1.0%	92.3%	96.0%
					2	0.03105	0.34512	36.07986	1.6%	1.9%	96.0%	101.4%
					3	0.02553	0.19801	34.79725	1.2%	1.2%	89.7%	93.3%
	TCA Crash Supernatant (Without Stabilizing Agent)	High QC Penicillamine	40.2	40.2	1	1.90317	0.06047	0.07647	93.1%	0.3%	0.2%	94.0%
					2	1.80814	0.76268	0.39572	90.6%	4.2%	1.1%	100.1%
					3	1.91785	0.24395	0.40665	93.9%	1.4%	1.0%	97.8%
		High QC Penicillamine-Penicillamine Disulfide	20.1	40.2	1	0.02911	17.78868	0.00245	1.4%	101.8%	0.0%	102.5%
					2	0.02498	17.89045	0.00698	1.3%	99.2%	0.0%	99.8%
					3	0.02784	17.49861	0.00705	1.4%	103.6%	0.0%	104.3%
		High QC Pencillamine-Cysteine Disulfide	40.2	40.2	1	0.02084	0.19664	39.38169	1.0%	1.1%	97.7%	100.9%
					2	0.01696	0.18877	39.29316	0.8%	1.0%	104.6%	107.5%
					3	0.01620	0.19022	40.69383	0.8%	1.1%	104.9%	108.0%
Comparison (0h)	N/AP (Fresh & With Stabilizing Agent)	High QC Penicillamine	40.2	40.2	1	2.04347			N/AP	N/AP	N/AP	N/AP
					2	1.99530						
					3	2.04279						
		High QC Penicillamine-Penicillamine Disulfide	20.1	40.2	1		17.47141					
					2		18.04235					
					3		16.89285					
		High QC Pencillamine-Cysteine Disulfide	40.2	40.2	1			40.32439				
					2			37.57015				
					3			38.77458				

Table 2: Short-Term Stability of Penicillamine, Penicillamine-Penicillamine Disulfide and Penicillamine-Cysteine Disulfide in Plasma and TCA Crash Supernatant Without stabilizing agent.

B. An Analytical Method That Measures Copper-Penicillamine Provides a More Accurate Measure of Therapeutic Penicillamine in the Bloodstream

Penicillamine is a chelating agent recommended for the removal of excess copper in patients with Wilson's disease. Copper is an essential trace element vital to the health of all

living organisms. For a healthy individual having body weight of 100 kg, copper levels may be as high as 10 mg of copper within the blood circulation. *In vitro* studies indicate that one atom of copper combines with two molecules of Penicillamine. So, for example, 210 mg of copper circulating in blood will form a chelate with approximately one gram (specifically 1025 mg) of Penicillamine.

The conversion of Penicillamine to Penicillamine-copper complex is very rapid (less than 30 mins) *in vitro*. Published literature also supports the rapid formation of Penicillamine-copper complex *in vivo* after administration of Penicillamine. [2] The excretion of Penicillamine-copper complex is reported as 1% excretion out of the total complex present in blood. [3] Therefore, a high percentage (99%) of Penicillamine-copper complex is present in the blood at the time of blood sample collection.

Given the rapid conversion to Penicillamine-copper complex both *in vivo* and *in vitro*, a more accurate measure of therapeutic penicillamine is the measure of free penicillamine plus penicillamine from the Penicillamine-copper complex. Current methods, including the TCA crash method used in the NDA for the Cuprimine RLD (and other ANDAs), do not include steps to measure penicillamine present in the Penicillamine-copper complex during the bioanalytical assay method. In contrast, the method adopted by Apicore in ANDA No. 210646 measures accurately the amount of Penicillamine from the Penicillamine-copper complex by cleaving the Penicillamine from the complex.

Table 3 shows LC/MS/MS data from the Apicore bioanalytical method to determine the conversion rates to Penicillamine of (1) Penicillamine-copper chelate; (2) Penicillamine-cysteine disulfide conjugate; and (3) Penicillamine-cysteine conjugate. Data in **Table 3** show that 95% of Penicillamine-copper chelate gets converted to free Penicillamine. Therefore, measuring Penicillamine from the Penicillamine-copper chelate is a more accurate quantification of the amount of penicillamine (as opposed to estimating from the disulfides).

[2] See, e.g., Birker, P. and Hans C. Freeman, "Structure, Properties, and Function of a Copper(1)-Copper(11) Complex of D-Penicillamine," Journal of the American Chemical Society 99:21, 6890-99 (1977) (Attachment 2).
[3] See Label of the Reference Listed Drug, Cuprimine (Penicillamine) 250 mg Capsules at p. 1 (Attachment 3).

Compound	Concentration in plasma (ng/ mL)	Conjugate Molar Ratio Vs Penicillamine	Average Area Ratio	CV	Conversion
Penicillamine - Cysteine Disulfide	14400	1.0: 1. 0	0.973	3.0%	82.7%
Penicillamine Copper complex	12500	0.5: 1.0	0.558	5%	95%
Penicillamine	8000	No Conjugate	1.176	2.1%	NA
Penicillamine and Penicillamine - Cysteine Disulfide	8000(penicillamine) and 14400(penicillamine – cysteine)	1.0: 1.0	2.056	4.7%	74.8%

Table 3: LC/MS/MS data to determine conversion of Penicillamine conjugate to Penicillamine using the Apicore method

i. The Presence of an Oxidizing Agent Does Not Affect Formation of Penicillamine-Albumin Conjugate

More than 80% of plasma Penicillamine is bound to proteins, especially albumin. These compounds containing a thiol functional group that usually are difficult to assay due to the rapid oxidation of the thiol group to the respective disulfide. The formation of Penicillamine-albumin conjugate shows that the formation is a difficult chemical conversion. Based on the crystal data, there is a loose interaction of albumin to Penicillamine and no strong covalent bond between them. Due to this weak interaction, the Penicillamine-albumin conjugate gets cleaved easily by most bioanalytical methods, including Apicore's method (see ANDA No. 210646) during protein precipitation, liquid-liquid extraction or solid phase extraction steps.

The addition of ferric chloride to enhance the oxidation of albumin and Penicillamine to form an albumin-Penicillamine conjugate results instead in the formation of Penicillamine disulfide. This indicates that the oxidation of Penicillamine to Penicillamine disulfide is much faster than the albumin-Penicillamine oxidation.

Table 4 contains HPLC (high performance liquid chromatography) data for the reaction of albumin and Penicillamine in the absence of an oxidizing agent. The data confirms there is no formation of Penicillamine-albumin conjugate.

Peak Results				
	Name	**RT**	**Area**	**% Area**
1	EDTA	1.67	848050	7.65
2	Peak2	1.86	17685	0.16
3	Peak3	2.26	22081	0.20
4	Peak4	3.11	3687	0.03
5	Penicillamine	3.89	9985571	90.09
6	Peak6	5.20	206826	1.87
SUM			11083899	100.00

Table 4: HPLC data for the reaction of Albumin and Penicillamine in absence of any oxidizing agents

A similar result is found when an oxidizing agent is used. **Table 5** contains HPLC data for the reaction of Albumin and Penicillamine in presence of ferric chloride, an oxidizing agent. The data show that the Penicillamine disulfide is formed rapidly, and there is no formation of the albumin-Penicillamine conjugate.

Name	**RT**	**PEN + Albumin + $FeCl_3$**	**PEN + Albumin + Excess $FeCl_3$**
Unknown peak	5.06	14.42	21.22
Penicillamine disulfide	***5.14***	***6.42***	***16.02***
Penicillamine	5.74	74.71	59.99
Unknown peak	20.11	0.22	0.13

Table 5: HPLC data for the reaction of Albumin and Penicillamine in presence of an oxidizing agent ferric *chloride in vitro*

The albumin-Penicillamine conjugate is a loosely bound supra molecular or Van Der Waals type of interaction, which can be easily cleaved. Apicore's current method, which uses 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB) as a derivatization agent that reacts with penicillamine through thiol-disulfide exchange, is able to cleave the weak albumin-Penicillamine conjugate and measure the amount of free Penicillamine.

ii. <u>The Presence of an Oxidizing Agent Does Not Affect Formation of Penicillamine-Cysteine Conjugate</u>

Penicillamine also is used for the treatment for patients with Cystinuria, a disease that is characterized by high concentrations of the amino acid cysteine in the urine, by eliminating excess Cysteine. The elimination is done, at least in part, by disulfide bond formation between

Penicillamine and cysteine, resulting in formation of Penicillamine-cysteine disulfide, a substance that is more soluble than Cystine and is excreted readily. Cysteine-Penicillamine conjugate is rapidly excreted from the body.[4]

The formation of Cysteine-Penicillamine conjugate is a very slow process, and it takes nearly eight days to get even 30% conversion of Cysteine to Cysteine –Penicillamine disulfide *in vitro*.

Sr. No.	Time (Hr.)	Cysteine Penicillamine disulfide (%)	Unreacted Penicillamine (%)	Unreacted cysteine (%)
1.	12	0.4	32.4	65.2
2.	24	3.5	22.1	58.6
3.	48	9.5	20.2	52.8
4.	72	20.3	16.3	40.6
5.	96	26.1	12.7	32.1
6.	120	30.6	11.2	27.3
7.	144	32.6	9.4	23.5
8.	168	33.3	8.48	21.2
9.	192	35.5	6.9	18.0

Table 6: In-vitro reaction times vs. concentration of Cysteine Penicillamine disulfide (%) formed without the presence of Catalyst (oxidizing agent)

The reaction rate is marginally better in the presence of an oxidizing agent like ferric chloride, but a conversion of only about 34% could be achieved with different concentrations (high enough to catalyze the reaction) of ferric chloride solution. After reaching approximately 34% formation of Cysteine-Penicillamine conjugate, the reaction seizes and does not progress further.

[4] See Label of the Reference Listed Drug, Cuprimine (Penicillamine) 250 mg Capsules at p. 1 (Attachment 3).

Sr. No.	1.0% $FeCl_3$ Solution,				0.5% $FeCl_3$ Solution,			
	Time (Hr.)	Cysteine Penicillamine disulfide (%)	Unreacted Penicillamine (%)	Unreacted cysteine (%)	Time (Hr.)	Cysteine Penicillamine disulfide (%)	Unreacted Penicillamine (%)	Unreacted cysteine (%)
1.	3	27.28	22.25	16.99	0.4	5.73	27.75	53.13
2.	4	30.91	20.01	12.62	2	18.21	21.26	42.79
3.	5	34.14	22.61	9.68	3	15.26	22.38	36.92

Table 7: Reaction rate of conversion of Cysteine to Cysteine –Penicillamine disulfide in presence of an oxidizing agent

The 1% concentration of ferric chloride used to form the Cysteine Penicillamine conjugate *in vitro* is a very high concentration that is not found *in vivo*. The contribution of free Penicillamine from the Cysteine–Penicillamine conjugate therefore is not significant with respect to the total free Penicillamine measured.

C. Current Methods for Measuring Therapeutic Penicillamine Do Not Accurately Measure Therapeutic Penicillamine Because They Do Not Account for Degradation After Sample Collection or Measure Penicillamine from the Penicillamine-Copper Complex

Current methods, such as the TCA crash method used in the Cuprimine RLD NDA, do not measure accurately the free Penicillamine at the time of blood collection because they do not employ steps to minimize oxidation of penicillamine, nor do they measure the amount of Penicillamine by cleaving Penicillamine from the Penicillamine-copper complex.

Current bioanalytical assays for Penicillamine indicate blood collection followed by plasma harvesting and treatment with TCA. The blood sample is either analyzed immediately, or the TCA supernatant is frozen until further analysis. In the published literature, penicillamine supernatant treated with HCl has shown stability when freeze-dried, but not when it is frozen at -20°C. The published literature is not clear on stability data from blood collection to plasma harvesting and/or long-term stability in plasma or supernatant.

As explained in Section III.A, the oxidation of Penicillamine that occurs after sample collection is non-reversible. To measure accurately the amount of Penicillamine at the time of blood collection, an analytical method needs to account for the oxidation of Penicillamine and arrest the degradation of Penicillamine into Penicillamine-disulfides. Existing methods do neither.

Existing methods also do not estimate the contribution of Penicillamine from Penicillamine-copper complex, yet the contribution of free Penicillamine from the copper complex is significant as explained in Section III.B. Apicore's method (see ANDA No. 210646) uses a series of reagents and stabilizers in a sequential way to protect the compound from in-vitro

oxidation to disulfide and at the same time cleave the Penicillamine from Penicillamine-copper complex.

For the above reasons, the FDA should approved ANDAs (or ANDA supplements) that use a bioanalytical method that introduces a stabilizing agent to minimize the oxidation of Penicillamine after sample collection, and measures free penicillamine from Penicillamine-copper complex, along with the circulating free Penicillamine (not bound to copper). Current methods do not account for stabilization, nor the release of copper-bound Penicillamine, and therefore present a risk that a proposed ANDA product does not meet bioequivalence standards, and hence does not have the therapeutic benefit described in the labeled claims.

III. Environmental Impact

This petition qualifies for a categorical exemption from the requirement to submit an environmental assessment under 21 C.F.R. §§ 25.30(h) and 25.31(a).

IV. Economic Impact

Pursuant to 21 C.F.R. § 10.30(b), economic impact information will be submitted upon request of the Commissioner following review of the petition.

V. Certification

I certify that, to my best knowledge and belief: (a) this petition includes all information and views upon which the petition relies; (b) this petition includes representative data and/or information known to the petitioner which are unfavorable to the petition; and (c) I have taken reasonable steps to ensure that any representative data and/or information which are unfavorable to the petition were disclosed to me. I further certify that the information upon which I have based the action requested herein first became known to the party on whose behalf this petition is submitted on or about the following date: October 10, 2019. If I received or expect to receive payments, including cash and other forms of consideration, to file this information or its contents, I received or expect to receive those payments from the following persons or organizations: Apicore. I verify under penalty of perjury that the foregoing is true and correct as of the date of the submission of this petition.

Sincerely,

Allison Fulton
SHEPPARD, MULLIN, RICHTER & HAMPTON LLP

References (List of Attachments):

1. Netter P. et. al., "Clinical Pharmacokinetics of Penicillamine-D," Clinical Pharmacokinetics 13, 317-333 (1987).
2. Webb et. al., "Identification of factors limiting the accurate measurement of plasma D-penicillamine in rheumatoid arthritis patients," Ann Clin Biochem 25: 186-191 (1988) (Attachment 2).
3. Birker, P. and Hans C. Freeman, "Structure, Properties, and Function of a Copper(1)-Copper(11) Complex of D-Penicillamine," Journal of the American Chemical Society 99:21, 6890-99 (1977).
4. Label of the Reference Listed Drug, Cuprimine (Penicillamine) 250 Capsules (Source: FDA database, Drugs@FDA: FDA Approved Drug Products).