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Usnic Acid Derivate from *Usnea sp.* and Bioactivity against *Arthemia salina* Leach

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Abstract. Usnic acid is chemical compound commonly found in lichen. These derivatives compounds both from the synthesis and from the isolation of various lichens have been studied as antibacterial, antifungal, antiparasitic, antiviral, and anticancer. Usnat acid derivative with not hydroxyl group on carbon C-3 was isolated from lichen usnea sp. taken from the Sinjaiselatan described in this paper. Its bioactivity against *Arthemia Salina* Leach. LC_{50} value of 19.49 μg/mL.

Introduction

Usnic acid is a lichen metabolite was first isolated in 1844 and it's still studied because it has a high commercial value. Pharmacological research at the beginning of the 1950s antibiotic era proposed usnic acid as an active compound [1]. Further investigation shows usnat acid as a lichen secondary metabolite which has many interesting bioactivity properties for industrial and health purposes, especially those related to human diseases caused by microbes. Unique biological and physiological properties make these compounds and their derivatives have potential applications in clinical pharmacology [2]. Usnic acid is found in lichen, especially in the *Usnea*, and also in Lichen *Alectoria*, *Cladonia, Lecanora, Ramalina* and *Evernia* [3]. Compounds that are closely related to usnat acid are also found in fungi such as *phytotoxin mycousnine* from the *Mycosphaerella nawae* fungus [4] and *cercosporamide* and *usnic acid amide* from the *Cercosporidium henningsii* [5].

Usnic acid (2,6-diacetyl-7,9-dihydroxy-8,9b-dimethyl-1,3 (2H, 9bH) -dibenzo-furandione) is a general chemical component found in lichen cells and is a chemical component of secondary metabolites main finding in all species of *Usnea sp.* From the basic structure of the molecule, this compound is found in the form of a pair of enantiomers as optically active namely; (-) - Usnic acid and (+) - Usnic acid. Physically the two enantiomers have the same physical properties and basic structure as the molecular formula $C_{18}H_{16}O_7$, it is very easy to form yellow crystals with needle or prism forms, having a melting point of around 203-204 ºC. Both enantiomers are distinguished from optical rotation which rotates the plane of right and left polarization depending on the polarization of the rotating plane of the methyl group on carbon 9b with each specific optical rotation $[\alpha]_D^{20}$= 495º and -495º [6]. In the practice of testing the results of isolation are not known for the mixture composition of the two. Different compositions or at a pure level will provide different biological activities [7].

Metabolism of usnic acid is known to be included in the *dibezofurano* group biosynthesis pathway from polyketide through acetyl coenzyme A then cyclization to form *methylphloracetophenone* then dimerization with the C1 bonding then aromatization involving *stereospecific oxidative phenolic coupling* of two units *methylphloracetophenone* hydrated and then dehydrated to ether formation produce usnic acid. The relevance of the molecular structure changes in functional groups and derivatives of usnat acid has been validated and further explored in the investigation of the many biological properties for medicinal purposes demonstrating the broad biological and physiological activity relevant in the field of clinical pharmacology with antimicrobial activity pathogenic to humans and plants. It's showed antiviral, antiprotozoal, antiproliferative, anti-inflammatory and

analgesic activity also showed ecological effects such as grouter, antiherbivorous and insecticidal properties. Differences in biological activity in some cases have been observed between two forms of enantiomer usic acid. Recently health food supplements containing usic acid have been promoted for use in weight reduction, with little scientific support. The current review emphasis is on the chemical and biological activity of usic acid and its derivatives in addition to rational and ecologically acceptable methods for the supply of these natural compounds on a large scale [8].

Usnic acid as a pure substance has been applied in the form of cream formulations, toothpastes, mouthwashes, sunscreen products, and some cases as preservatives. At present, a review of usnic acid's biological and biological activities is needed and its derivatives are rational and can be accepted ecologically to provide usnic acid on a large scale [8]. Lichen as the main source of the discovery of usnic acid is widely found growing in the Sinjaiborong area of Sinjaiselatan district, South Sulawesi province, especially in high altitude areas with low humidity. In this study, usnea sp. obtained from the area, which is one of the lichen species found in many trees and branches of coffee plants. 1,8-diaminooctane compounds as derivative of usnic acid were evaluated on murine and human cancer cell lines, It showed significant cytotoxicity against L1210 cancer cells [9]. Therefore, usnat acid derivative it found in this research was tested for citotoxy against *Arthemia salina* as a preliminary test for anticancer correlation.

Experimental

The plant material is usnea sp. taken from Sinjai Borong, South Sulawesi Province, Indonesia. Specimens were identified in Herbarium Bogoriensis LIPI Bogor document number FR-7.5.1PU.01-02.Ed. Organic solvents (E. Merck) n-hexane, chloroform, ethyl acetate, acetone, and methanol. Silica gel TLC plate GF_{254}, G 60 silica gel (70-230 mesh), catalog 1.07734, and $CeSO_4$ 10%, in 2N sulfuric acid. A total of 500 g of *usnea sp.* dry macerated with 6.5 liter chloroform previously with n-hexane, the obtained macerate was filtered and evaporated obtained 16 g extract. Chloroform extract was fractionated with chromatography column with the stationary phase are silica gel and mobile phase with n-hexane, ethyl acetate and methanol with gradient. Fractionation carryout six fraction (A-F). Continuously fractionating of D (2.54 g) with chromatography column, and with hexane: chloroform: ethyl acetate (2: 7: 1) as mobile phase and then crystallization of D1 obtained compound **2** (34 mg). Compound 2 was measured by melting point with *kruss* M5000 melting point, elucidation of the structure by Shimadzu FT-IR spectrophotometer prestige-21 KBr plate, 500NMR spectrometry Agilent (^1H at 500 MHz and ^{13}C at 125 MHz). *Brine Shrimp Lethality Test* (BST) follows BN Meyer's procedure [10].

Results and Discussion

Structure elucidation; Compound 2 (Fig. 1) obtained as solid yellow crystals, melting point 198-200ºC. IR (KBr) *v* (cm^{-1}) 3438, 2918, 1708, 1614, and 1136. IR spectrum gives absorption wave numbers (ν, cm^{-1}) ν = 3438 cm^{-1} as the OH group, ν = 2918 cm^{-1} as the asymmetric stretching vibration CH, ν = 1707 cm^{-1} as the vibration of the carbonyl group C=O, ν = 1614 cm^{-1} is vibration of the C=C bond from aromatics, ν = 1136 cm^{-1} as vibration of the COC. The spectrum representing the functional group in usnic acid.

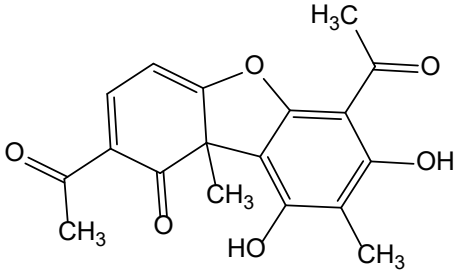

Figure 1. Structure of compound 2

The spectrum of ^{1}H-NMR (Fig. 2) shows proton signals are indicating seven proton namely; four aliphatic protons δ_H (1.76 ppm (3H, s); 2.10 ppm (3H, s); 2.66 ppm (3H, s) and 2.68 ppm (3H,s) respectively as methyl proton at position 9b-CH3, H8-CH_3, H-2-OC$\underline{C}H_3$ and 6-OC$\underline{C}H_3$. An aromatic proton δ_H 5.98 ppm (1H, s) at position 4, as well as two hydroxyl proton attached to the aromatic ring δ_H (13.31ppm (1H, s) and 11:02 ppm (1H, s). The ^{13}C-NMR spectrum showed 18 carbon signal; six aromatic carbon δ_C (155.34; 101.66; 164.02; 109.46; 157.64; 104.09), four-carbon olefin δ_C (105.37; 191.84; 98.46 ; 179.50), three carbonyl carbon δ_C (198.18; 201.89; 200.44), four aliphatic carbon methyl δ_C (28.03; 31.41; 7.68; 32.26), and one quaternary carbon δ_C (59.22).

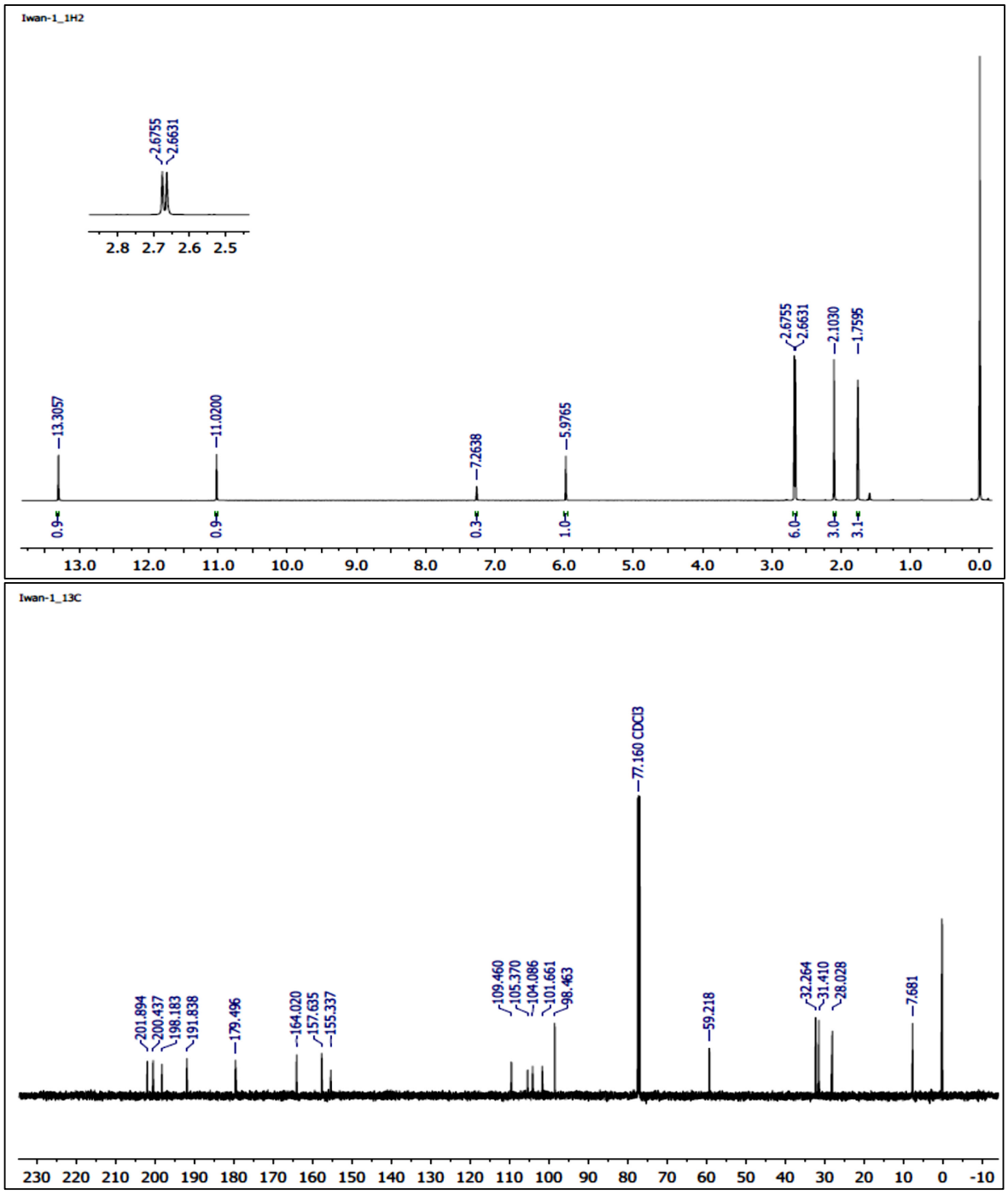

Figure 2. Spectrum ^{1}H-NMR and ^{13}C-NMR of Compound 2

Carbon framework and the location of each proton are shown from the HMBC and HMQC correlation (Fig. 3). All correlation of HMBC, is shown below; proton 2-OC$\underline{CH_3}$ δH = 2.66 (s) with carbon C-2 (δC = 105.37), 2-$\underline{C}$= O (δC = 201.89). Proton 4H δH = 5.98 (s) with carbon C3 (δC = 191.84), C4 (δC = 98.46), and C9b (δC = 59.22). Proton 6-OCC$\underline{H_3}$ δH = 2.68 (s) with carbon 6-C$\underline{CO}$ (δC = 200.44), C-6 (δC = 101.66). Proton 7-OH δH = 13.31 (s) with carbon C7 (δC = 164.02), C6 (δC = 101.66), C8 (δC = 109.46). Proton 9b-$\underline{CH_3}$ δH = 1.76 with carbon C1 (δC = 198.18), C9b (δC = 59.22), C9a (δC = 104.09), and C4 (δC = 98.46). Proton 8-$\underline{CH_3}$ δH = 7.68 with carbon C8 (δC = 109.46), C9 (δC = 157.64), C7 (δC = 164.02). Proton 9-OH δH = 11.02 with carbon C9 (δC = 157.64), C9b (δC = 59.22), C8 (δC = 109.46). HMQC showed that the proton correlation with carbon are below; proton (A) 9b-$\underline{CH_3}$ (δH = 1.7) with C9b-C carbon δH = 59.22; proton (B) 8-$\underline{CH_3}$ (δH = 2.10) with carbon C8-$\underline{CH_3}$ δH = 7.68; proton (C) 6-OC$\underline{CH_3}$ (δH = 2.68) with carbon 6-O$\underline{C}$ δH = 200.44; proton (D) 2-OC$\underline{CH_3}$ (δH = 2.66) with carbon 2-O$\underline{C}$ δH = 201.89 ppm).

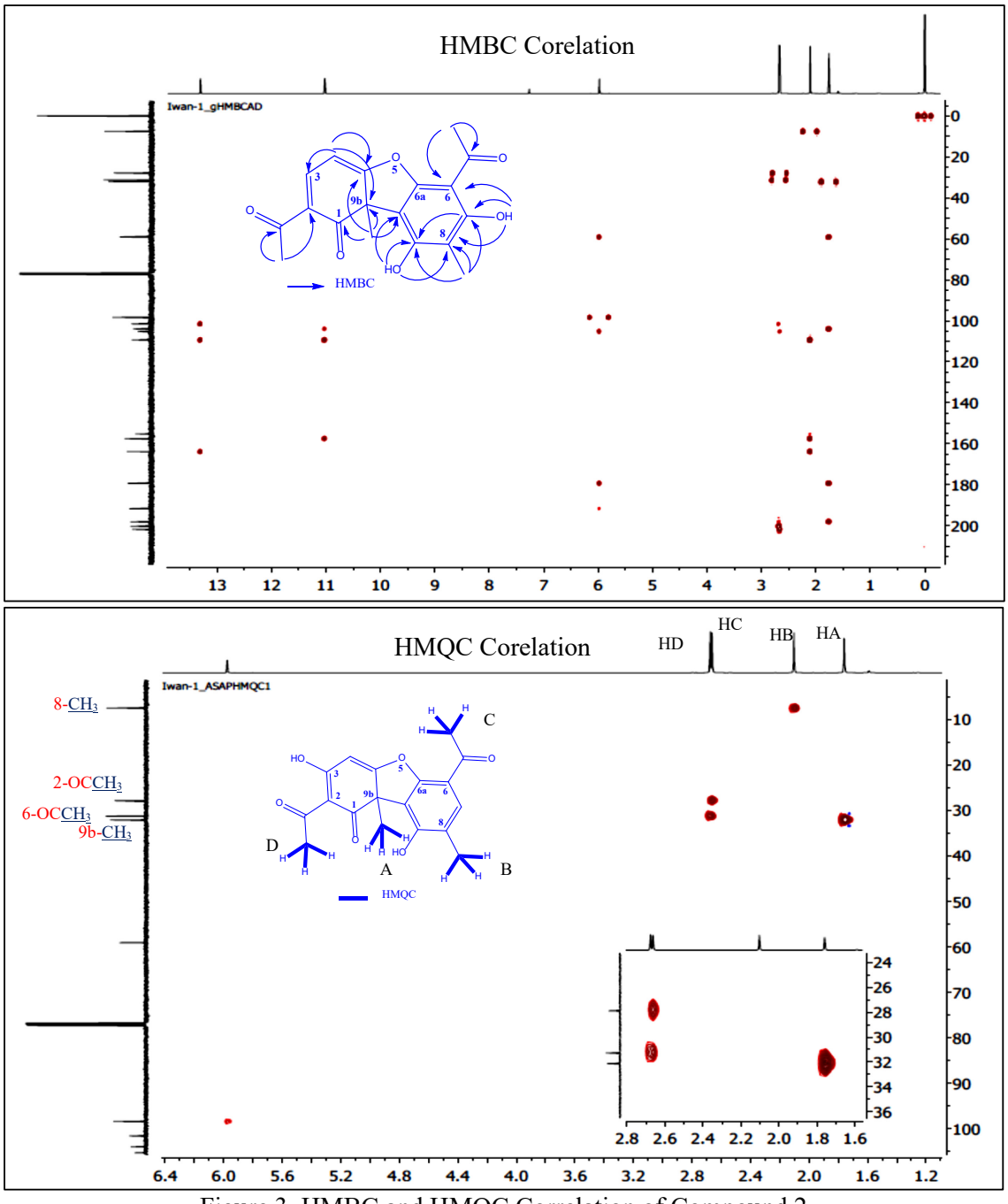

Figure 3. HMBC and HMQC Correlation of Compound 2

Based interpretation of ^{1}H-NMR, ^{13}C-NMR, NMR-2D, IR, data and by comparing NMR spectrum data of usnic acid [11], the compound (1) was ascertained as usnat acid derivative (molecular structure as shown in Figure 1) which is minus the hydroxyl group on the C-3 atom because there is no aliphatic hydroxyl observed at chemical shift ≥ 17 ppm.

Bioactivity against *Arthemia Salina* Leach

Based on the LC_{50} cytotoxicity BST against *A. Salina*, the compound 2 be considered as moderately toxic considering to the 50% of lethality it caused at the concentration of 19.49 µg/mL, the data calculate showed in Fig. 4 and Table 1. This criterion is in agreement with the American National Cancer Institute that fixed the limitation of IC_{50} must be lower than 30µg/mL. The compound 2 as moderately toxic it have correlation as anticancer [11]. Synthesis of several usnic acid derivate has catalytic activity of tyrosyl-DNA phosphodiesterase enzyme with IC_{50} 0.33-2.7 µM and low cytotoxicity to human cells. That derivate can be used for anticancer therapies that are more effective when combined with topoisomerase 1 inhibitors [12].

Table 1. The Data %death of *A. Salina* Leach. versus concentration of compound 2

Con. (ppm)	log con.	Number of *A. Salina* Leach				% death		% death corrected	probity
		Compound 2		Control		sample	control		
		first	death	first	death				
15.62	1.19	28.00	16.00	20.0	0.0	57.14	0.00	57.14	5.18
31.25	1.49	28.00	18.00	21.0	1.0	64.29	4.76	62.50	5.33
62.50	1.80	30.00	22.00	21.0	2.0	73.33	9.52	70.53	5.55
125.00	2.10	27.00	22.00	23.0	3.0	81.48	13.04	78.70	5.81
250.00	2.40	25.00	22.00	20.0	4.0	88.00	20.00	85.00	6.04
500.00	2.70	36.00	32.00	24.0	5.0	88.89	20.83	85.96	6.08
1000.00	3.00	35.00	35.00	22.0	8.0	100.00	36.36	100.00	8.09

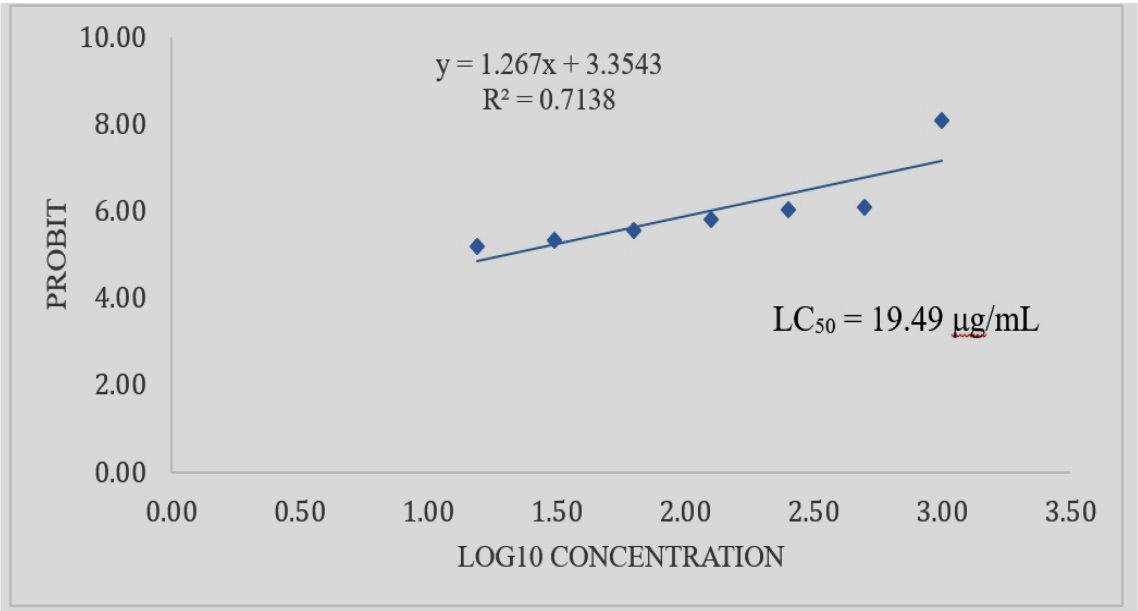

Figure 4. Graph of probity %lethality versus $\log_{10}$ concentration for chronic (24 hours) toxicities of compound 2

Summary

Based on the results of research that concluded that the usnat acid derivate have been isolated from the chloroform extract of *usnea sp.* which has no hydroxyl group on the carbon C3. The compound dissolved in DMSO 5% concentration showed cytotoxic properties against *Arthemia Salina* Leach. LC_{50} value of 19.49 μg/mL.

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