

AD-A285 351



DOT/FAA/AM-94/16

Office of Aviation Medicine
Washington, D.C. 20591

**Elimination of Quinine in
Two Subjects After
Ingestion of Tonic Water:
An Exploratory Study**

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August 1994

Final Report

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94-31505



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Technical Report Documentation Page

1. Report No. DOT/FAA/AM-94/16		2. Government Accession No.		3. Recipient's Catalog No.	
4. Title and Subtitle Elimination of Quinine in Two Subjects After Ingestion of Tonic Water: An Exploratory Study				5. Report Date August 1994	
7. Author(s) Vicky L. White, Dennis V. Canfield, Ph.D., and Jerry R. Hordinsky, M.D.				8. Performing Organization Report No. NTIS DTIC	
9. Performing Organization Name and Address FAA Civil Aeromedical Institute P.O. Box 25082 Oklahoma City, OK 73125				10. Work Unit No. (TRAIS) J...	
12. Sponsoring Agency name and Address Office of Aviation Medicine Federal Aviation Administration 800 Independence Avenue, S.W. Washington, D.C. 20591				11. Contract or Grant No. By	
15. Supplemental Notes This work was performed under Task AM-B-91-TOX-66				13. Type of Report and Period Covered / Availability Codes Avail and/or Special	
16. Abstract Biological specimens from 8 fatal aviation accidents out of 775 fatal aviation accidents analyzed in 1991 and 1992 were found to contain quinine. In one case, the investigators sought to identify the source of quinine found in the pilot. It was suggested that the quinine might have come from the consumption of tonic water. Since no recent use of quinine or tonic water could be found, the investigators asked how long quinine could be detected in a urine specimen. A limited research project was undertaken to provide a preliminary range of the approximate length of time quinine could be detected in urine and blood. Each of 2 male subjects was given a 20 oz. bottle of tonic water, which contained 35 mg of quinine. Quinine was detected using standard laboratory TLC and HPLC methods. Quinine has such diverse applications as a treatment for muscle cramps and malaria, in addition to being an additive in tonic water. Since adverse effects have been identified at plasma concentrations between 10-15 µg/mL, no performance effects would be expected from the maximum concentrations of quinine found (0.291 µg/mL) in this study after the ingestion of one 20 oz. bottle of tonic water. However, based on this study, the possibility of prolonged detection (over 8 days) of quinine should (a) serve as a warning against using this as a sign of recent use of quinine directly or in association with alcohol, and (b) alert the investigators to inquire about disorders or conditions that might impair performance, but for which quinine treatment was terminated days before the accident.				14. Sponsoring Agency Code A-1	
17. Key Words Tonic water, Quinine			18. Distribution Statement Document is available to the public through the National Technical Information Service, Springfield, Virginia, 22161		
19. Security Classif. (of this report) Unclassified		20. Security Classif. (of this page) Unclassified		21. No. of Pages 10	22. Price

ELIMINATION OF QUININE IN TWO SUBJECTS AFTER INGESTION OF TONIC WATER: AN EXPLORATORY STUDY

INTRODUCTION

Eight fatal aviation accident victims out of 775 fatal aviation accident victims analyzed in 1991 and 1992 were found to contain quinine. In one case, the accident investigators sought to identify the source of quinine found in the pilot. It was suggested that the quinine may have come from the consumption of tonic water mixed with alcohol. Since no recent use of quinine or tonic water could be found, the investigators asked how long quinine could be detected in a urine specimen. A limited research project was undertaken to establish the approximate length of time quinine could be detected in urine and blood.

The use of quinine was first reported in 1633 by an Augustinian monk (5). The drug is used for the treatment of malaria and muscle cramps, as a diluent in heroin, and as an additive in tonic water. Toxic levels of this drug range from 10-15 μ g/mL in plasma. In acute overdosage, this drug has been known to cause severe ocular, cardiovascular, gastrointestinal, and central nervous system toxicity (4). Therapeutic levels of quinine range from 3 to 7 μ g/mL in plasma (2). Patients with hypersensitivity to quinine have been known to suffer from tinnitus, impaired hearing, headache, visual disturbances, and nausea at therapeutic levels. The maximum amount of quinine allowed, under law, in tonic water is 80 μ g/mL.

The molecular structure of quinine can be seen in Figure 1. This molecular structure results in a high absorption coefficient of 959 in aqueous acid solutions (2). This high absorption coefficient makes it possible to detect and identify quinine by HPLC at sub-therapeutic levels in both blood and urine.

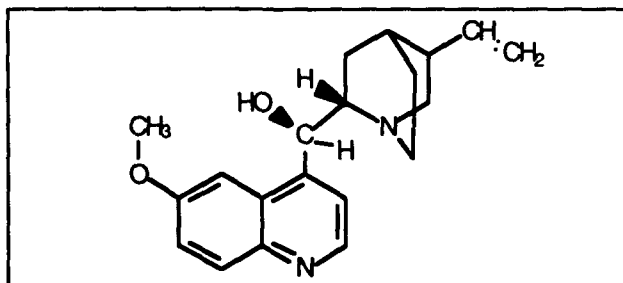


Figure 1. Quinine molecular structure

METHOD

Two male subjects were each given a 20oz bottle of tonic water that contained 35mg of quinine at approximately 08:00 hours. Subject #1 was a 50 year-old male and weighed 280 lbs. (127.1 kg). Subject #2 was a 37 year-old male and weighed 182 lbs. (82.6kg). Food and liquid intake were not controlled during the experiment. Urine was sampled as follows in subject #1: every 30 mins for 2 hours; every hour for the next 6 hours, every normal void for the next 3 days; and randomly for the next 4 days. Subject #2 was sampled at each normal urination over a period of 27 hours. Blood was drawn from each subject at approximately 2.5, 8.5, and 24 hours.

Quinine was detected using standard laboratory Thin Layer Chromatography and High Performance Liquid Chromatography methodologies. The TOXILAB A base procedure was used to extract and screen all specimens for quinine. A Hewlett Packard 1090 II HPLC with diode array detector linked to a ChemStation, and a Lichrospher 60 RP-select B 3 μ m, 250 x 4mm column was used in the confirmation and quantitation process of quinine. The HPLC procedure utilized was reported by Logan (1). The HPLC was run at 1.3mL/min and the peaks detected at 240nm were scanned from 190.0nm to 400.0nm (Fig. 2) using an HP diode array detector. The HPLC oven temperature was set for 35°C. A 5 μ L sample was injected using an HP auto-injector. Quinine HCL and flurazepam HCL standards were obtained from Alltech Applied Science Lab. The methanol and acetonitrile were Burdick and Jackson HPLC grade obtained from a commercial vendor. In this experiment, flurazepam was used as an internal standard because it has a relatively high absorption coefficient at the wave length being monitored and has similar extraction properties; base line resolution was obtained when mixed with quinine (Fig. 3). Working standards were diluted into negative blood and urine to establish a calibration curve. A sufficient amount of internal standard was then added to 5mls of blood and urine to give 700ng/mL internal standard in blood

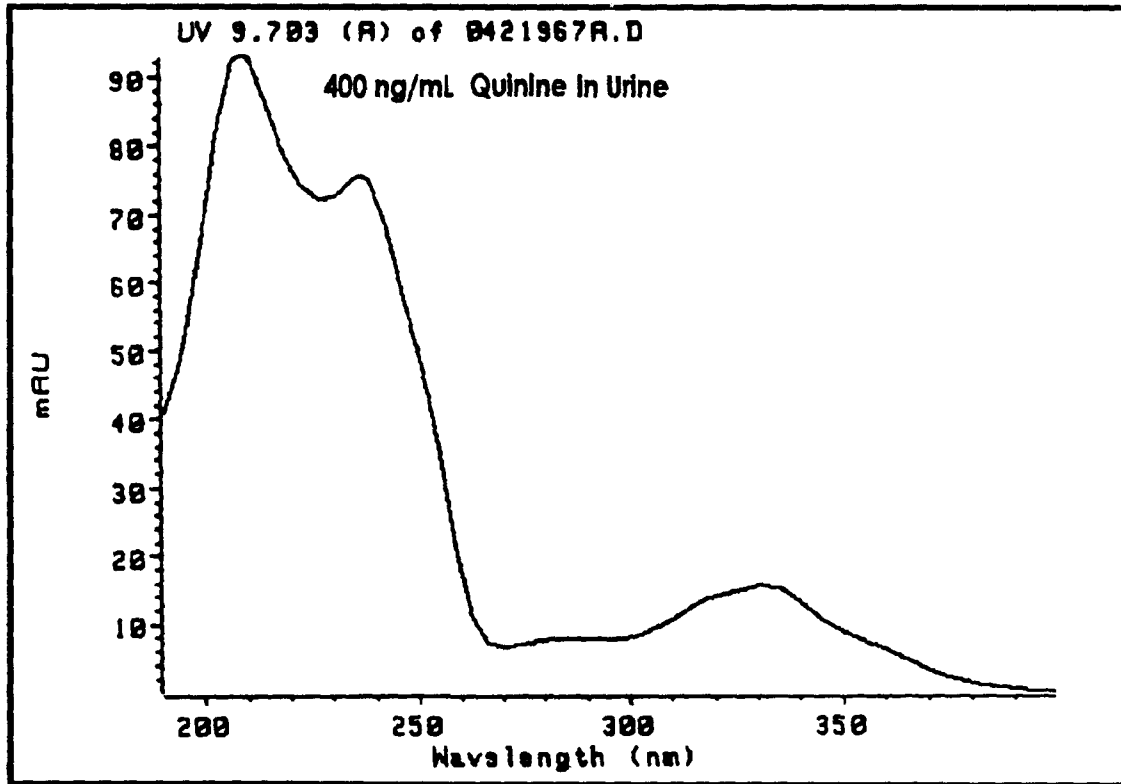


Figure 2. UV spectra of quinine

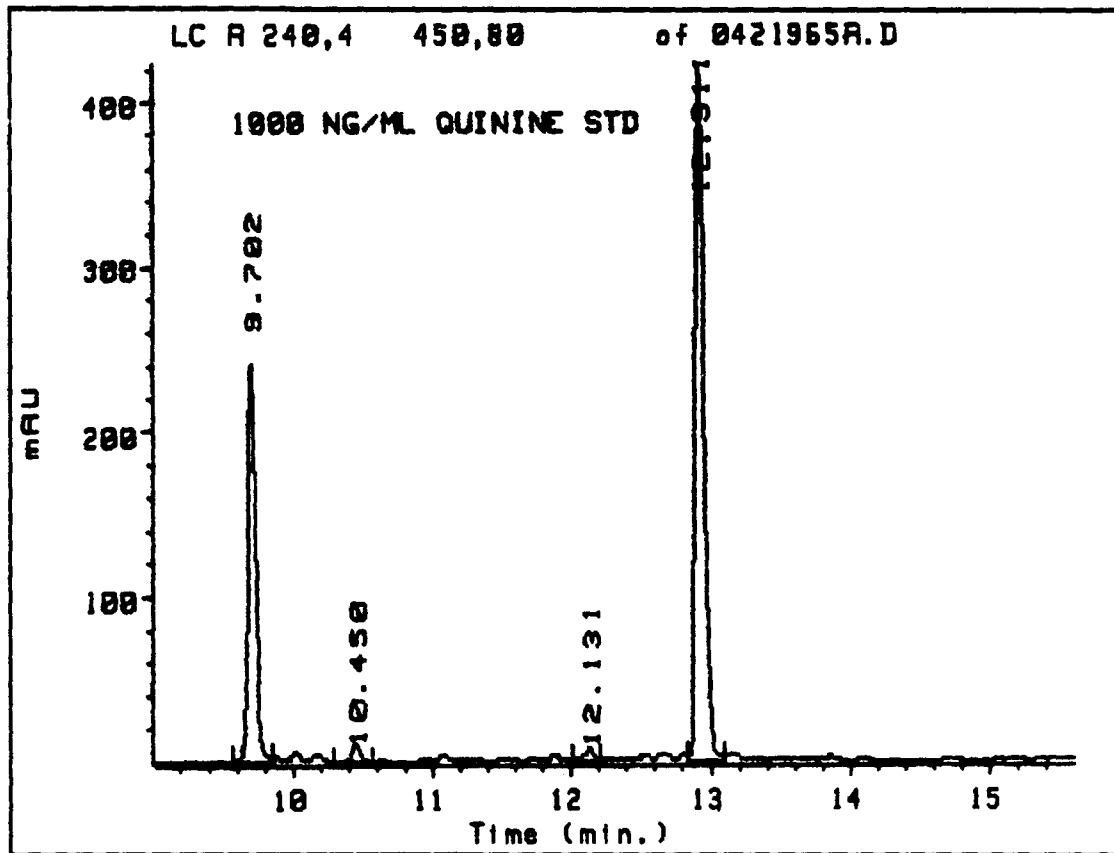


Figure 3. HPLC Chromatograph of quinine and the internal standard.

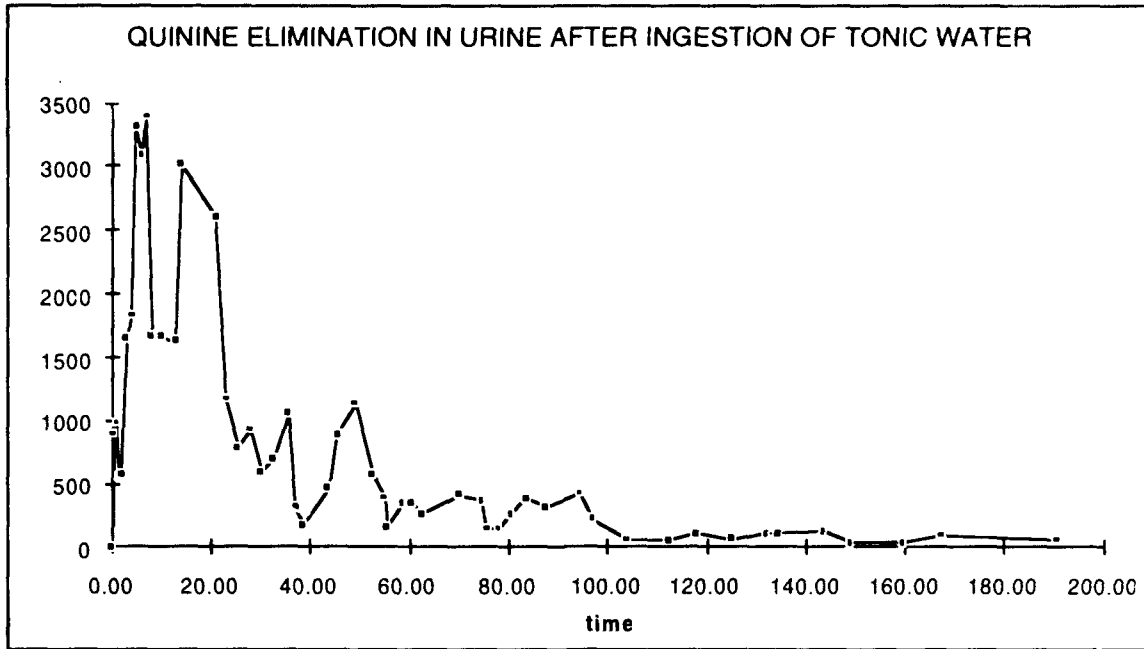


Figure 4. Graphic representation of time versus concentration of quinine in urine for subject #1.

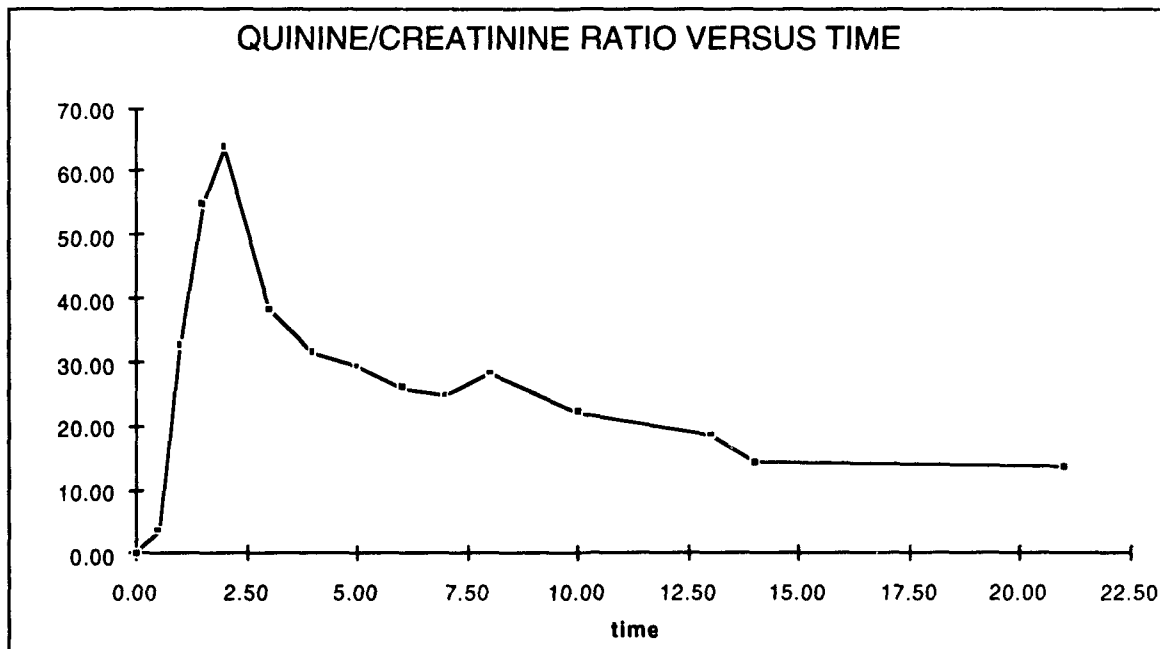


Figure 5. Graphic representation of time versus the ratio of quinine/creatinine in urine for subject #1.

and 2500ng/mL internal standard in urine. The urine samples were mixed on a rotator for 5 mins. The blood proteins were precipitated by adding acetonitrile and then mixed on a rotator. The blood samples were then centrifuged at 2000 rpm for 10 minutes, and the liquid portion was decanted into a beaker and evaporated under a fume hood until the odor of acetonitrile was not detected. The aqueous layer remaining after the evaporation of acetonitrile was then treated in the same way as urine samples for extraction with TOXI-LAB tubes. The TOXI-LAB tubes were then centrifuged for 5 minutes, and the organic layer was placed into a silanized glass conical centrifuge tube, and evaporated until dry, under a gentle stream of nitrogen in an N-EVAP. The tubes were reconstituted with 50 μ L methanol for the urine extracts, and 25 μ L for the blood extracts. 5 μ L was injected into the HPLC.

Creatinine levels were measured in urine using an Abbott TDx fluorescence polarization immunoassay instrument. The creatinine was used to normalize the concentration of quinine in urine. The quinine concentration was divided by the creatinine level found in the urine. This representation of the time versus quinine curve compensates for variation in sample volume and sampling intervals and provides a curve in units of time versus quinine/creatinine ratio (Fig. 5).

RESULTS

The tonic water used in this experiment was found to contain 59 μ g/mL of quinine. The highest recorded urine level was in subject #2, who reached a maximum urine level of 3.91 μ g/mL of quinine (Table 2) 9 hours after drinking 20 ounces (592mL) of tonic water containing approximately 35mg of quinine. Both subjects obtained a maximum concentration of quinine in blood at approximately 2 to 3 hours after ingestion of the tonic water (Tables 3 and 4). The highest recorded blood level was in subject #2 who reached a maximum concentration of 0.29 μ g/mL of quinine in blood. After 24 hours, the blood quinine

level had dropped to 0.184 μ g/mL in subject #2 (Table 4). The experiment was terminated at 8 days, at which point the urine in subject #1 still demonstrated quinine, albeit at a very low level of 0.058 μ g/mL (Table 1).

DISCUSSION AND CONCLUSIONS

Clearly the maximum concentration of quinine in blood and urine is affected by the weight of the subject. If the concentration of quinine in the blood of subject #2 at 2.25 hours is adjusted for the weight difference between the two subjects ($182/280 \times 0.291\mu\text{g/mL}$ quinine), the calculated level of 0.189 μ g/mL of quinine is approximately the same as the 0.187 μ g/mL found in subject #1 at the same time.

As would be expected, there is wide variation in the concentration of quinine in urine with changes in time, as demonstrated over the 8-day observation period for subject #1 (Fig. 4). This variation, which is caused by deviations in the times between collection and the changes in total body water content, can be compensated for by plotting the ratio of quinine to creatinine versus time (Fig. 5). This curve shows a concentration maximum at approximately 2.5 hours, which is consistent with what is found in blood (4) and does not show wide fluctuations in concentration after the first 4 hours.

Adverse effects are normally identified at plasma concentrations between 10-15 μ g/mL; therefore, no performance effects would be expected from the maximum concentration of quinine found (0.291 μ g/mL) after the ingestion of one 20 oz. bottle of tonic water. However, the possibility of prolonged detection of quinine should (a) serve as a warning against using this as a sign of recent use of quinine directly or in association with alcohol, and (b) alert the investigators to inquire about disorders or conditions that might impair performance, but for which a quinine prescription was terminated days before the accident.

Table 1. Table of time, quinine (Q) levels, creatinine (C) levels, and ratios for urine specimens collected from subject #1. Times are reported in half-hour intervals.

Time Interval	Quinine ng/ml	Creatinine mg/dL	Q/C Ratio	Day	Actual Time
0.00	0	NA	0.00	1	08:06
0.50	894	247	3.62		08:49
1.00	978	30	32.60		09:22
1.50	548	10	54.80		09:45
2.00	574	9	63.78		10:15
3.00	1644	43	38.23		11:15
4.00	1826	58	31.48		12:20
5.00	3312	113	29.31		13:20
6.00	3083	119	25.91		14:30
7.00	3390	137	24.74		15:30
8.00	1667	59	28.25		16:30
10.00	1658	75	22.11		18:20
13.00	1628	88	18.50		21:10
14.00	3010	211	14.27		22:20
21.00	2602	192	13.55	2	05:45
23.00	1172	96	12.21		07:45
25.50	783	66	11.86		10:05
28.00	926	89	10.40		12:40
30.00	589	54	10.91		14:20
32.50	698	79	8.84		17:00
35.50	1054	112	9.41		20:00
37.00	308	46	6.70		21:20
38.50	178	31	5.74		22:50
43.50	461	66	6.98	3	04:00
45.50	887	97	9.14		06:00
49.00	1131	111	10.19		09:24
52.50	575	94	6.12		13:15
55.00	387	65	5.95		15:20
55.50	160	30	5.33		16:05
58.50	338	76	4.45		19:05
60.50	338	91	3.71		21:03
62.50	263	125	2.10		23:03
70.00	410	136	3.01	4	06:22
74.50	368	154	2.39		09:50
75.50	146	76	1.92		11:10
78.00	146	83	1.76		13:20
80.50	260	111	2.34		15:45
83.50	385	183	2.10		19:00
87.50	313	189	1.66		23:07
94.50	423	223	1.90	5	06:10
97.00	222	117	1.90		08:30
104.00	57	81	0.70		15:25
112.50	50	72	0.69		24:00
118.00	103	100	1.03	6	05:20
125.00	60	92	0.65		12:20
132.00	108	123	0.88		19:22
134.50	104	132	0.79		22:15
143.50	120	229	0.52	7	07:00
149.00	38	123	0.31		12:45
159.50	34	162	0.21		22:46
167.50	92	271	0.34	8	07:00
190.50	58	271	0.21	9	05:45

Table 2. Table of time, quinine (Q) levels, creatinine (C) levels, and ratios for urine specimens collected from subject #2. Times are reported in half-hour intervals.

Time Interval	Quinine ng/mL	Creatinine mg/dL	Q/C Ratio	Day	Actual Time
0.00	0	127	0.00	1	08:00
1.50	454	27	16.81		09:30
3.00	1307	42	31.12		11:00
9.00	3912	164	23.85		17:00
10.50	1630	136	11.99		18:30
14.75	1781	147	12.12		22:45
19.50	755	50	15.10	2	03:30
22.00	524	40	13.10		06:00
24.00	775	73	10.62		08:00
27.25	671	75	8.95		11:15

Table 3. Table of time, quinine levels for blood specimens collected from subject #1.

Time Interval	Quinine ng/mL	Actual Time
0.00	0	08:06
2.25	187	10:15
8.25	118	16:15
24.00	57	08:00

Table 4. Table of time, quinine levels for blood specimens collected from subject #2.

Time Interval	Quinine ng/mL	Actual Time
0.00	0	08:00
2.50	291	10:30
8.25	217	16:15
23.50	184	07:30

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