氏　　名	高 山 成 明
生 年 月 日	
本　　籍	石川県
学 位 の 種 類	博士（薬学）
学 位 記 番 号	博乙第175号
学位授与の日付	平成10年9月30日
学位授与の要件	論文博士（学位規則第4条第2項）
学位授与の題目	Studies on Analyses of Stimulants in Human Urine and Hair Samples Using HPLC/Chemiluminescence Detection Method （化学発光検出高速液体クロマトグラフィを用いたヒト尿及び毛髪からの覚せい剤分析に関する研究）
論文審査委員	（ 主 査 ） 早川 和一 （ 副 査 ） 木津 良一，辻　　彰，太田 富久，中島憲一郎

学 位 論 文 要 旨

Abstract

An HPLC/chemiluminescence detection (HPLC/CL) method of analyzing methamphetamine (MA) and its metabolites, which is most frequently abused among illegal drugs, was developed, and the analysis was performed using the urine and hair samples from MA addicts. The following results were obtained. First, MA and its metabolites including glucuronide conjugates were detected in as small as 2 ml of urine, and this method could reveal that present ratio of MA and its metabolites in the urine of MA addicts. Second, this method allowed detection of MA and its major metabolite, amphetamine (AP), using only a single hair, and was shown to be an excellent analytical method when collection of a large quantity of hair was difficult or their contents in hair were low. Third, hair was shown to be an excellent sample for proving drug use from discussion of the results of analysis of hair and urine samples together with the situation of MA use. Fourth, since this analysis could be performed using only a single hair, black hair and white hair of addicts could be separately analyzed, and it was found that MA and AP contents of white hair were lower than black hair, suggesting the affinity of these compounds with melanin. Fifth, it was demonstrated that the contents of MA and AP decreased after permanent wave, dye

and decolorant treatments of natural black hair of addicts containing these compounds.

Chapter 1. Determination of MA, AP and piperidine in human urine

Determination of MA, AP and piperidine in human urine has been developed. The three compounds, extracted into diethyl ether from alkaline urine, were derivatized with dansyl chloride, then separated on a reversed-phase column and chemiluminogenically detected after reaction with bis(2,4,6-trichlorophenyl)oxalate (TCPO) and hydrogen peroxide. The corresponding peaks obtained from human urine were identified as the dansyl derivatives by mass spectrometry. MA levels as low as 37 pg/ml in urine were determined. The sensitivity of the method is higher than that of Simon's reagent test and gas chromatography. (Refer to Fig. 1)

Chapter 2. Determination of MA, AP and other metabolites in urine

MA, AP and other metabolites (norephedrine (NE), p-hydroxymethamphetamine (pOHMA), p-hydroxyamphetamine (pOHAP)) were derivatized with dansyl chloride. They were separated on a reversed phase column with gradient elution using an acetonitrile – tetrahydrofuran – imidazole buffer mobile phase and chemiluminogenically determined using TCPO and hydrogen peroxide as post column reagents. AP, NE and pOHAP were derivatized with naphthalene-2,3-dicarboxaldehyde, and were separated on a reversed phase column using an acetonitrile – imidazole buffer mobile phase and chemilumigenically determined. Enzymatic hydrolysis of glucuronide conjugates (G) allowed them to be determined as pOHMA and pOHAP, respectively. Utilizing the two methods, MA and all metabolites were determined in urine samples of MA addicts. The tendency, in order of decreasing concentration was: [MA] > [AP] > [pOHMAG] > [pOHMA] > [NE] > [pOHAPG] > [pOHAP]. Although ephedrine was detected in several samples, it was not considered to be a metabolite of MA but rather a component derived from cough medicine. (Refer to Fig. 2)

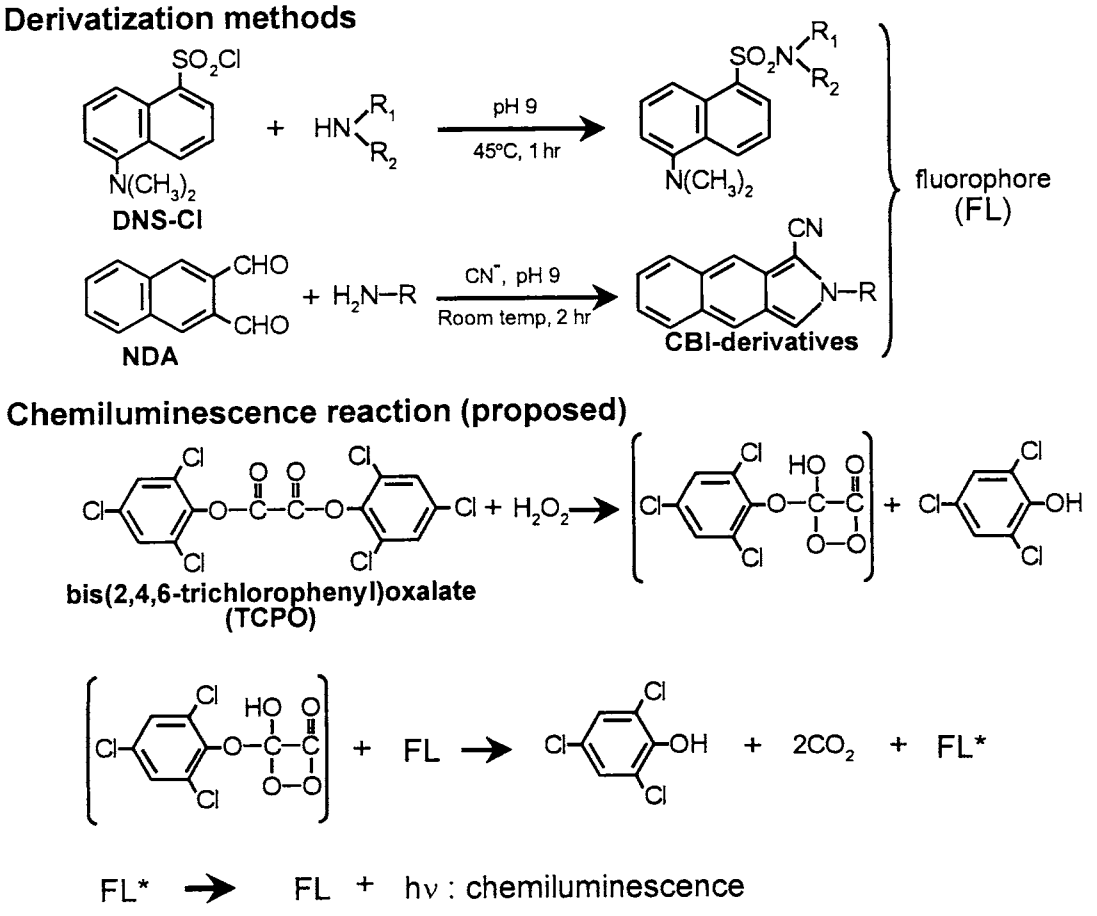

Fig. 1. The mechanisms of derivatization and chemiluminescence reaction

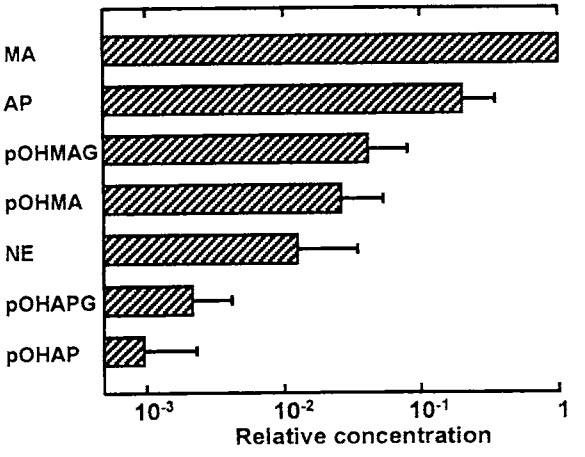

Fig. 2. Relative concentrations of MA metabolites in addict urine samples

Each column and horizontal bar represent the mean and S.D. of the nine samples.

Chapter 3. Determination of MA and AP in a single hair

Determination of MA and AP in a single human hair sample has been developed in which TCPO and hydrogen peroxide are the post column reagents. After washing a single hair sample with water and methanol, it was cut into pieces, and extracted with a mixed solution of methanol and hydrochloric acid for 1 h under ultra-sonication and allowed to stand at room temperature overnight. Then the organic phase was evaporated to dryness. To the residues, 0.1 ml of carbonate buffer and 0.1ml of dansyl chloride solution were added and the solution was heated at 45°C for 1 h. An aliquot of the reaction mixture was then subjected to HPLC/CL. MA and AP were chemiluminogenically detected as their dansyl derivatives from only a single hair sample. The detection limit was about 2 pg in an injected volume (20 μl), and about 20 pg in a single hair sample. This detection limit was smaller than that by GC/MS/SIM

Table 1. MA and AP concentrations in hair samples

Sample No.	Days *		Hair **			Urine***
	urine	hair	MA ng/mg	AP ng/mg	AP/MA	
1	1	7	24.4 ± 3.20	1.64 ± 0.29	0.07	D
2	1	5	4.46 ± 1.42	0.88 ± 0.75	0.20	D
3	1	3	5.13 ± 4.43	0.40 ± 0.28	0.08	D
4	3	3	27.6 ± 15.3	2.28 ± 0.25	0.08	D
5	1	1	27.3 ± 9.36	3.02 ± 0.87	0.11	D
6	4	4	4.40 ± 3.19	0.16 ± 0.07	0.04	D
7	1	7	6.71 ± 3.89	1.37 ± 0.44	0.20	D
8	4	25	21.9 ± 5.49	1.34 ± 0.34	0.06	D
9	2	25	0.53 ± 0.33	0.20 ± 0.08	0.38	D
10	1	16	0.27 ± 0.19	0.10 ± 0.05	0.37	D
11	1	11	9.80 ± 1.41	0.80 ± 0.14	0.08	D
12	2	16	19.5 ± 0.70	5.50 ± 1.41	0.28	D
13	1	30	7.30 ± 0.71	0.90 ± 0.07	0.12	D
14	2	4	1.37 ± 0.50	0.45 ± 0.07	0.33	D
15	2	9	8.80 ± 3.75	1.00 ± 0.36	0.11	D
16	2	2	0.33 ± 0.15	0.08 ± 0.02	0.24	D
17	1	2	6.88 ± 4.99	1.05 ± 0.45	0.15	D
18	3	9	1.86 ± 0.81	0.33 ± 0.05	0.18	D
19	3	20	89.7 ± 22.6	9.22 ± 4.46	0.10	D
20	1	21	29.0 ± 4.24	3.20 ± 0.81	0.11	D
21	3	21	12.8 ± 6.06	2.39 ± 1.14	0.19	D
22	un	(2)	25.2 ± 5.86	2.46 ± 0.64	0.10	D
23	un	(2)	23.5 ± 12.1	2.87 ± 1.06	0.12	D
24	1	20	3.81 ± 1.77	ND		D
25	1	1	2.71 ± 0.43	ND		D
26	9	43	0.40 ± 0.29	ND		D
27	2	2	1.38 ± 0.56	ND		D
28	1	10	7.81 ± 2.73	ND		D
29	3	13	ND	ND		D
30	2	16	ND	ND		D
31	2	24	ND	ND		D
32	16	16	0.77 ± 0.27	0.24 ± 0.09	0.31	ND
33	52	60	4.81 ± 2.53	1.18 ± 0.34	0.24	ND
34	13	17	2.50 ± 1.29	1.05 ± 0.35	0.42	ND
35	10	10	1.64 ± 0.41	0.40 ± 0.28	0.24	ND
36	15	20	3.17 ± 1.75	0.93 ± 0.35	0.29	ND
37	6	20	0.47 ± 0.21	0.12 ± 0.09	0.26	ND
38	7	17	6.28 ± 1.37	0.59 ± 0.19	0.09	ND
39	10	18	0.21 ± 0.09	ND		ND
40	7	8	0.15 ± 0.13	ND		ND
41	5	8	1.74 ± 0.80	ND		ND
42	120	120	ND	ND		ND
43	20	20	ND	ND		ND
44	-	23	0.30 ± 0.07	0.10 ± 0.03	0.33	-

*, days between last use and sampling (urine and hair) (2), 2 days after urine sampling; -, no sample.
**, mean ± SD; n=2 – 10; ND, not detected.
***, D, both MA and AP were detected; ND, neither MA nor AP was detected; -, not analyzed.

method.

Chapter 4. Determination of MA and AP in hair from 44 MA users

Determination of MA and AP in hair samples obtained from 44 MA users. Of these, 31 samples showed both MA and AP, 8 samples showed only MA and 5 samples showed neither MA nor AP. Seven MA users whose hair samples contained both MA and AP, neither compound was detected in the urine. Because the present method can be performed on a single hair, it is easier to procure samples and to obtain the subjects' willingness to provide samples. (Refer to Table 1 and Fig. 3)

Chapter 5. Determination of MA and AP in black and white hair

Black and white hair samples were obtained from black-, gray- (*i.e.*, a mixture of black and white hair) and white-haired MA users, and MA and AP were determined. MA and AP were detected in black hair, which were contained in the part of the hair that grew in the period of MA use. In the same subjects, MA concentrations were lower in white hair than those in black hair. AP was not detected in white hair. This

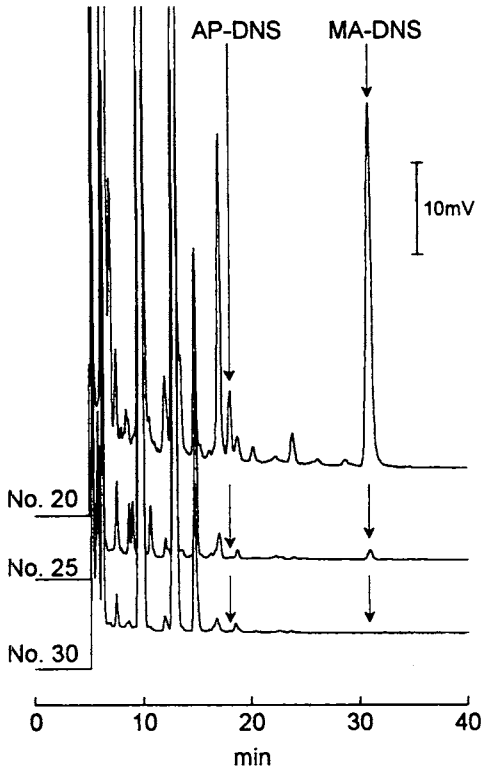

Fig. 3. Typical chromatograms of extracts of hair samples

Table 2. Results of hair analyses of black and white hairs

Sample No.	Black Hair		White Hair	
	MA ng/mg	AP ng/mg	MA ng/mg	AP ng/mg
1	1.37 ± 0.50 (*n*=3)	0.51 (*n*=1) 0.40 (*n*=1) ND (*n*=1)	0.50 (*n*=1) 0.39 (*n*=1) trace (*n*=1)	ND (*n*=3)
2	89.7 ± 22.6 (*n*=5)	9.22 ± 4.46 (*n*=5)	24.4 (*n*=1) 15.0 (*n*=1)	ND (*n*=2)
3	10.2 ± 4.86 (*n*=5)	1.88 ± 0.97 (*n*=5)	0.20 (*n*=1) trace (*n*=3) ND (*n*=1)	ND (*n*=5)
4	23.5 ± 12.1 (*n*=5)	2.87 ± 1.06 (*n*=5)	1.10 (*n*=1) 0.40 (*n*=1) trace (*n*=2) ND (*n*=1)	ND (*n*=5)
5	4.81 ± 2.53 (*n*=5)	1.18 ± 0.34 (*n*=5)	ND (*n*=3)	ND (*n*=3)
6	ns	ns	ND (*n*=5)	ND (*n*=5)

ns, no hair sample, ND, not detected.

difference may be related to an affinity of MA and AP with melanin. (Refer to Table 2)

Chapter 6. Effects of permanent wave, dye and decolorant treatments on MA and AP in hair

Black hairs that had been removed from a MA addict were treated with permanent wave, dye or decolorant treatments, respectively, and MA and AP were detected. The concentrations of MA and AP in the hair decreased significantly in all cases. In separate experiments, both MA and AP were found to be stable in the permanent wave treatment solutions, but not stable in the dye or decolorant treatment solutions. Therefore, MA and AP were eluted from hair in the permanent wave treatment solutions, and destroyed in dye or decolorant treatment solutions. These results suggested that treatments of permanent wave, dye or decolorant disturb determination of MA and AP from hair of MA addicts. (Refer to Fig. 4)

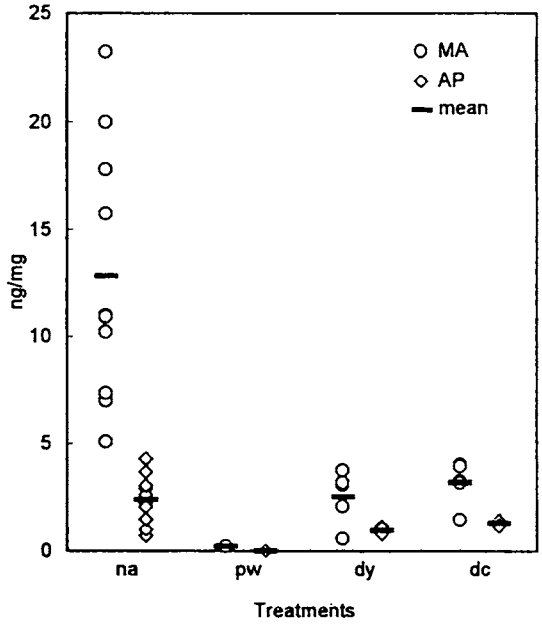

Fig. 4. Comparison of MA and AP concentrations of natural black hairs before and after permanent wave, dye or decolorant treatments
na, natural black hair; pw, permanent wave treated hair; dy, dye treated hair; dc, decolorant treated hair.

Chapter 7. Conclusions

In conclusion, the HPLC/CL method is very useful for proving the use of MA using the urine and hair samples, and is considered to be applicable to analysis of other drugs. Among them, it is considered to be readily applicable to analysis of a fluorescent psychedelic, lysergic acid diethylamide (LSD), and 3,4-methylenedioxymethamphetamine (MDMA) with structures similar to that of MA. In addition, since the result of hair analysis reveal previous intake status, this analysis is applicable to survey of long-term exposure to environmental pollutants that were taken unconsciously and this report was considered to be useful for such study.

学位論文審査結果の要旨

〔審査経過〕平成10年7月1日の第1回審査委員会で審査方針を決定した。まず基礎学力を確認し，各委員による面接と諮問を行い，8月5日の口頭発表を最終試験と定めた。口頭発表終了後第2回審査委員会を開催し，協議の結果以下のとおり判定した。

〔論文の主な内容〕乱用薬物覚せい剤（メタンフェタミン，MA）及びその代謝物の超高感度分析法を開発し，乱用者尿及び毛髪中のMAに関する詳細な検討を行った。(1) MAとその代謝物をダンシル誘導体化して過シュウ酸エステル化学発光検出高速液体クロマトグラフィーに適用する方法を開発し，尿に応用可能とした。(2) MA，アンフェタミン（AP），パラヒドロキシ（pOH）MA，ノルエフェドリン，pOHAP及びpOH体のグルクロン酸抱合体の濃度から，一般的日本人乱用者尿の特徴を明らかにした。(3)毛髪1本からのMA，AP検出を初めて可能とし，毛髪が尿よりMA使用の前歴を遡れることを示した。(4)日本人の黒髪と白髪ではMA，AP濃度に違いがあること，パーマネント，染色及び脱色処理がその濃度を減少させることを明らかにした。

〔審査結果〕本研究はMA及びその代謝物の超高感度分析法を開発し，尿及び毛髪に応用して多くの知見を提供している。科学捜査面でもMA乱用証明における本法の有用性を示している。さらに毛髪分析が環境汚染物質の長期暴露の解析にも有望なことを示唆している。以上を総合して，本審査委員会は本論文が博士（薬学）に値すると判定した。