CLIN. CHEM. 39/1, 104–108 (1993)

Analytical Performance Evaluation of EMIT® II Monoclonal Amphetamine/Methamphetamine Assay: More Specificity than EMIT® d.a.u.™ Monoclonal Amphetamine/Methamphetamine Assay

Amitava Dasgupta,[1] Sylvia Saldana,[1] George Kinnaman,[1] Muriel Smith,[2] and Kaaren Johansen[2]

We evaluated a new EMIT® II monoclonal amphetamine/methamphetamine assay for screening human urine by comparing it with the EMIT® d.a.u.™ monoclonal amphetamine/methamphetamine assay and a fluorescence polarization assay. The EMIT II assay has a cutoff of 1 mg/L *d*-methamphetamine. The EMIT II and EMIT d.a.u. assays were run on a BM/Hitachi 704 analyzer; for the fluorescence polarization assay we used a TDx analyzer. All EMIT II positive samples were also analyzed by the fluorescence polarization assay. We used gas chromatography/mass spectrometry (GC/MS) for confirmation of the presence of amphetamine or methamphetamine. Within-run CVs for the Level 1 (1 mg/L) and Level 2 (3 mg/L) calibrators for the EMIT II assay were 0.47% and 0.53%, respectively. Corresponding between-run CVs were 1.48% and 1.60%, respectively. Of the 1007 samples screened for amphetamines, 50 were positive by the EMIT d.a.u. assay; 21 samples (not a subset of the 50 samples) were positive by the EMIT II assay. However, 19 samples that tested positive by EMIT II also tested positive by the EMIT d.a.u. assay. Subsequent testing of the EMIT II positive samples by the fluorescence polarization assay detected in six positive samples. By means of chiral derivatization we identified two specimens containing primarily *l*-isomers of amphetamine and methamphetamine. Sympathomimetic amines were identified in several of the samples not containing amphetamine or methamphetamine.

Additional Keyphrases: *abused drugs · enzyme immunoassay · fluorescence polarization, GC/MS compared*

Amphetamines are central nervous system stimulants that produce alertness, wakefulness, increased energy, reduced hunger, and an overall feeling of well-being. *d*-Amphetamine, *d*-methamphetamine, and *d,l*-amphetamine are the most common amphetamines (*1*, *2*). Amphetamine is commonly marketed as a racemic mixture (*3*). Present clinical use is limited to the treatment of narcolepsy and attention deficit disorder. Tolerance and physiological dependency develop with large doses of amphetamines, leading to its abuse. As a central nervous system stimulant, *d*-amphetamine is two to four times more potent than *d,l*-amphetamine and has a much higher potential for abuse. Amphetamines can be taken orally, intravenously, by smoking, or by nasal inhalation (*2*). Metabolism occurs in the liver either by deamination or hydroxylation of the aromatic ring and (or) side chain. However, deamination is the major metabolic pathway in humans, with the metabolic end product hippuric acid being conjugated to glycine (*3*, *4*). Amphetamine is also excreted in the urine unchanged, whereas methamphetamine is partially metabolized to amphetamine. Amphetamines appear in the urine within 3 h after any type of administration and can be detected by the EMIT® assay (Syva Co., San Jose, CA) for as long as 24–48 h after the last dose (*2*).

Amphetamine induces intense stimulation of the central nervous system (*5*, *6*). Amphetamine-induced psychosis with euphoria and hallucination is common (*7*). Severe toxicity of amphetamine may lead to death (*8–10*).

Methods used for detecting amphetamine in biological fluids include thin-layer chromatography, gas chromatography, fluorometry, microcrystallography, radioimmunoassay, and enzyme immunoassay (*6*). Immunoassay methods are usually used in clinical laboratories for screening human urine (*11*). Such assays provide only a preliminary analytical result, however, and a more specific alternative chemical method must be used to obtain a confirmed analytical result. Gas chromatography/mass spectrometry (GC/MS) is the preferred confirmatory method.[3] In addition, chiral derivatization of amphetamines is useful in determining whether *d* or *l* isomers are present, because the *d* isomer has a higher potential for abuse. The EMIT d.a.u. monoclonal amphetamine/methamphetamine assay (EMIT d.a.u.) may produce an unconfirmed positive result with therapeutic doses of the following: chloropromazine, ranitidine, chloroquine, *N*-acetylprocainamide, phentermine, ephedrine, and phenylpropanolamine. We evaluated a new EMIT II monoclonal amphetamine/methamphetamine assay (EMIT II) for analytical performance and found that the new assay is more specific than the EMIT

[1] Clinical Laboratories, MC 0004, The University of Chicago Hospitals, 5841 S. Maryland Ave., Chicago, IL 60637.
[2] Syva Company, San Jose, CA.
Received March 18, 1992; accepted August 24, 1992.

[3] Nonstandard abbreviations: GC/MS, gas chromatography/mass spectroscopy; EMIT d.a.u., EMIT d.a.u. monoclonal amphetamine/methamphetamine assay; EMIT II, EMIT II monoclonal amphetamine/methamphetamine assay; NIDA, National Institute on Drug Abuse; MDA, methylenedioxyamphetamine; and MDMA, methylenedioxymethamphetamine.

d.a.u. now available for detecting amphetamine and methamphetamine.

Materials and Methods

EMIT II kits were provided for evaluation by Syva. EMIT d.a.u. kits were also obtained from Syva; fluorescence polarization assay kits were purchased from Abbott Laboratories (Abbott Park, IL). Both the EMIT II and EMIT d.a.u. were run on a BM/Hitachi 704 analyzer (Boehringer Mannheim, Indianapolis, IN) and the fluorescence polarization assay for amphetamine/methamphetamine was run on a TDx (Abbott Laboratories), analyzer. GC/MS analyses were performed on a Hewlett-Packard (Palo Alto, CA) Model 5890 gas chromatograph coupled to a Model 5970B mass selective detector.

The EMIT II is a homogeneous immunoassay with a cutoff of 1.0 mg/L for *d*-methamphetamine, which is consistent with the concentration recommended in the National Institute on Drug Abuse (NIDA) guidelines for screening of amphetamines in human urine (2). The assay also detects *d*-amphetamine (≥1.0 mg/L), *d,l*-amphetamine (≥1.5 mg/L), *l*-amphetamine (≥6 mg/L), methylenedioxyamphetamine (MDA; ≥3 mg/L), and methylenedioxymethamphetamine (MDMA; ≥6 mg/L).

The assay was run on a BM/Hitachi 704 analyzer by monitoring the change in absorbance at 340 nm in the final reaction mixture. Before each run, reconstituted reagents were allowed to equilibrate in the reagent compartment within the analyzer for 1 h, and the calibrators and controls were allowed to equilibrate at room temperature for 1 h. The Level 0 (negative) and Level 2 (3 mg/L) calibrators were assayed once and the Level 1 (1 mg/L) calibrator, in duplicate. The following controls were assayed once: 800 *A* and 1200 *A* *d*-methamphetamine, and 800 1200, and 1000 *A* *d*-amphetamine. After 50 urine samples were screened, the Level 1 calibrator was again assayed in duplicate as a drift check.

The specificity of all the immunoassays was evaluated by assaying a drug panel. Stock solutions of the drugs to be evaluated were added to a negative urine pool to a final concentration of 10 and 200 mg/L. The manufacturer's protocols were used for all cross-reactivity testings. Drugs producing a response above the cutoff value of the assay were considered positive.

For GC/MS confirmation of amphetamine and (or) methamphetamine in positive screening samples, the urine was adjusted to pH 9 and applied to a solid-phase extraction column (narc-1; J.T. Baker, Phillipsburg, NJ). The columns were washed with isopropanol/water (20:80 by vol); the drugs were then eluted with methanolic hydrochloric acid. After derivatization with heptafluorobutyric anhydride (Sigma Chemical Co., St. Louis, MO), the samples were injected onto the GC/MS. Standards and controls for GC/MS analysis were treated by the same protocol. Internal standards were *d*-amphetamine-d_3 and *d,l*-methamphetamine-d_5. A Hewlett-Packard Ultra II capillary column containing 5% phenyl methyl silicone (12 m × 0.2 mm × 0.33 μm) was used for GC/MS analysis. The initial oven temperature of the gas chromatograph was 90 °C; this temperature was maintained for 0.50 min after injection. Oven temperature was increased at 25 °C/min to 300 °C, which was maintained for 1.0 min. The mass spectrometer was operated in the single-ion-detection mode. For the identification of amphetamine, ions were monitored at *m/z* 118.0 and 240.2; for methamphetamine, ions were monitored at *m/z* 118.0, 210.0, and 254.3. Internal standards were identified by monitoring ions at *m/z* 121.0 and 243.2 for amphetamine-d_3 and *m/z* 258.3 for methamphetamine-d_5.

For the chiral derivatization, amphetamine and methamphetamine were extracted from alkaline urine samples with 1-butyl chloride and derivatized with *N*-trifluoroacetyl-L-prolyl chloride (0.1 mol/L in chloroform; Regis Chemical, Morton Grove, IL). We also used the Ultra II capillary column for this. Internal standards were *d,l*-methamphetamine-d_5 and *d,l*-amphetamine-d_3. The initial oven temperature, 175 °C, was maintained for 0.5 min after injection, then was increased at 11 °C/min to 275 °C, which was maintained for 1.0 min. Under these conditions for GC/MS analysis, *l*-methamphetamine elutes at 5.06 min, which is 0.15 min before elution of *d*-methamphetamine (retention time 5.21 min). Selected ion monitoring was focused on *m/z* 251 for *d*- and *l*-methamphetamine and on *m/z* 255 for the internal standard. Similarly, *l*-amphetamine elutes at 4.15 min, which is 0.17 min before *d*-amphetamine (retention time 4.32 min). The selected ion monitoring was focused on *m/z* 237 for *d*- and *l*-amphetamine and on *m/z* 241 for the corresponding internal standards. Mass spectral fragmentation patterns are given in Figure 1. The percentage of *d* isomer was calculated from the total of the mixed isomers.

Results and Discussion

Within-run CVs for the Level 1 and Level 2 calibrators with the EMIT II were 0.47% and 0.53%, respectively. The corresponding between-run precision CVs for the Level 1 and Level 2 calibrators were 1.48% and 1.60%, respectively. These results indicate very good precision of the EMIT II assay for amphetamine and methamphetamine.

A total of 1007 urine samples were screened for the presence of amphetamines by both the EMIT d.a.u. and EMIT II. Fifty samples were positive by the EMIT d.a.u., but only 21 samples by the EMIT II. Interestingly, 19 of these 21 samples were also positive by EMIT d.a.u. and the remaining 2 samples were negative by EMIT d.a.u. Only samples positive by EMIT II and EMIT d.a.u. were assayed further by the fluorescence polarization assay. Of the 21 samples positive by the EMIT II (Table 1), 6 were positive with the fluorescence polarization assay (*d*-amphetamine cutoff of 1.0 mg/L); 2 of these 6 samples (samples T and U) contained amphetamine and methamphetamine in concentrations above the NIDA-recommended GC/MS confirmation concentration (>0.5 mg/L methamphetamine and ≥0.2 mg/L amphetamine). Subsequent chiral derivatization of these two samples identified *l*-amphetamine and *l*-methamphetamine as the major portion of amphetamines present. Sample T contained *d*-amphetamine

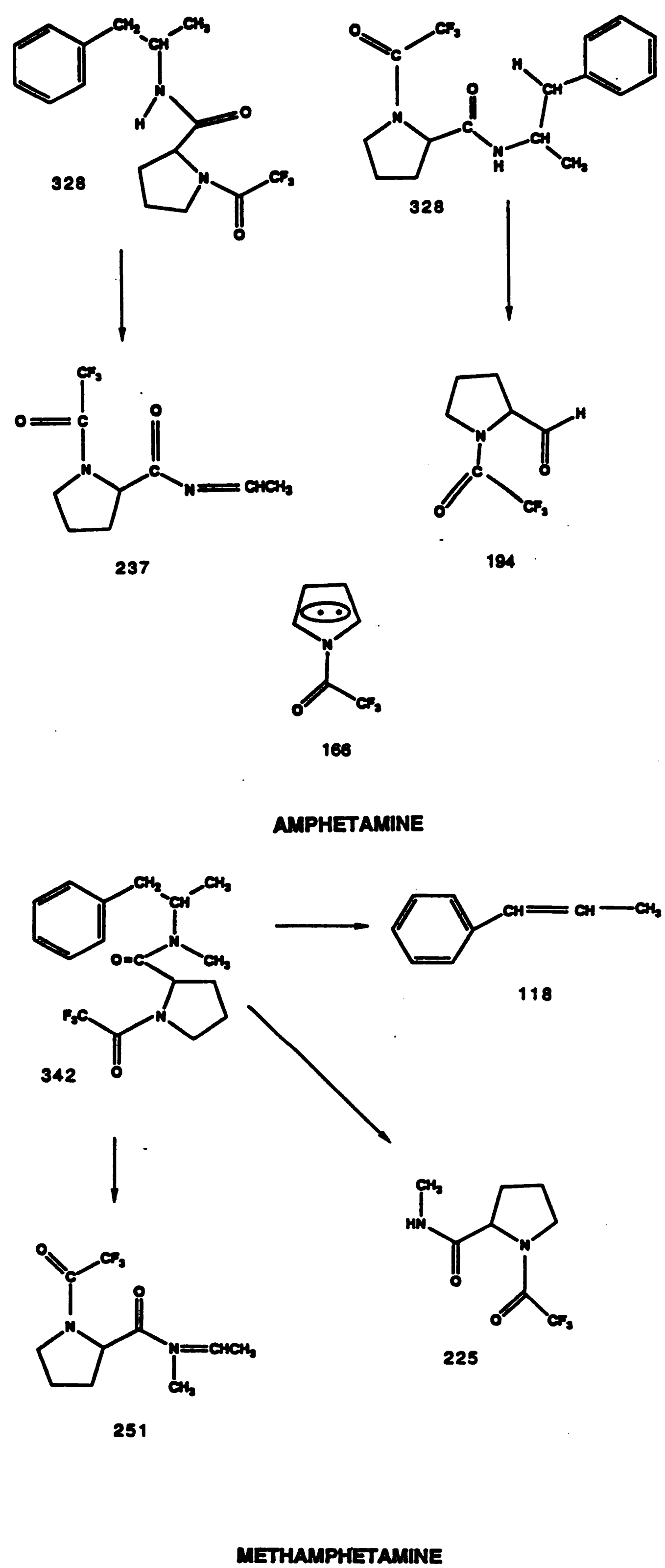

Fig. 1. Mass spectral fragmentation patterns of *N*-trifluoroacetyl-L-prolyl chloride derivatives of amphetamine and methamphetamine
Numbers indicate mass/charge ratios (*m/z*)

(0.16 mg/L), *l*-amphetamine (1.74 mg/L), *d*-methamphetamine (0.15 mg/L), and *l*-methamphetamine (2.72 mg/L). Sample U contained *d*-amphetamine (0.10 mg/L), *l*-amphetamine (1.19 mg/L), *d*-methamphetamine (0.12 mg/L) and *l*-methamphetamine (2.26 mg/L). GC/MS of these two samples did not detect the presence of any of the following sympathomimetic amines: ephedrine, pseudoephedrine, phentermine, or phenylpropanolamine. However, the presence of mainly *l*-amphetamine and *l*-methamphetamine in the specimens indicates that these samples were not positive for amphetamine abuse.

GC/MS analysis of 18 of the samples positive by EMIT II and EMIT d.a.u. samples (sample S was unavailable for GC/MS) identified one or more compounds, including ephedrine, pseudoephedrine, phentermine, and phenylpropanolamine in several samples. No amphetamine or methamphetamine was identified in these 18 samples (Table 1). These data indicate that both of the EMIT assays and fluorescence polarization exhibit some cross-reactivity with the above compounds. The cross-reactivity of these screening assays to other compounds, evaluated with a drug panel, is summarized in Table 2.

Data from this study can be used to determine the clinical sensitivity of these assays to a selected group of sympathomimetic amines. Several sympathomimetic amines were identified in 8 of the 18 positive EMIT II samples not containing significant quantities of amphetamine or methamphetamine. Other sympathomimetic amines not identified by GC/MS may have been responsible for the unconfirmed positive results in the 10 remaining samples. All of these samples were positive by both the EMIT II and EMIT d.a.u.

Four specimens (A, B, C, and D), positive by both of the EMIT assays and by the fluorescence polarization assay, contained phentermine at 1–10 mg/L; in addition, specimens A and B contained high concentrations of ephedrine and phenylpropanolamine. Two samples negative by fluorescence polarization (E and F) contained large quantities of phenylpropanolamine and pseudoephedrine, respectively, but no phentermine. Because the concentration of phenylpropanolamine in sample E exceeds that in sample B, these data suggest that cross-reactivity to phentermine is responsible for the unconfirmed fluorescence polarization results for sample B. Cross-reactivity data also support this conclusion. For the EMIT II and EMIT d.a.u., the data for sample E suggest that cross-reactivity to phenylpropanolamine is responsible for the unconfirmed EMIT screening results. Data from samples G and H indicate that trace concentrations of ephedrine and phentermine do not interfere with the fluorescence polarization assay. Sample F also contained a trace amount of *l*-methamphetamine (<0.3 mg/L) in addition to ephedrine, pseudoephedrine, and phenylpropanolamine. EMIT screening results for this sample were strongly positive by the EMIT d.a.u. and slightly positive by EMIT II. When NIDA criteria are used for GC/MS confirmation, this sample is negative.

Therefore, cross-reactivity to ephedrine, pseudoephedrine, phentermine, and phenylpropanolamine is responsible for the unconfirmed positive results with both of the EMIT assays for these samples. For the fluorescence polarization assay, cross-reactivity to phenter-

Table 1. GC/MS and Fluorescence Polarization Data: Samples Positive by the EMIT II[a]

Sample	Ephedrine	Pseudoephedrine	Phentermine	Phenylpropanolamine	Fluorescence polarization	EMIT II
	Concentration, mg/L					
	GC/MS					
A	>10	<1	5–10	ND	>8.0	155
B	ND	ND	1–5	5–10	>8.0	105
C	<0.3	ND	1–2	ND	7.92	74
D	ND	ND	1–2	ND	>8.0	69
E	ND	ND	ND	>10	0.03	22
F	<0.5	1–5	ND	<0.3	0.06	18
G	<0.5	ND	ND	ND	0.02	69
H	ND	ND	<0.3	ND	0.00	29
I	ND	ND	ND	ND	0.47	15
J	ND	ND	ND	ND	0.15	57
K	ND	ND	ND	ND	0.08	24
L	ND	ND	ND	ND	0.06	18
M	ND	ND	ND	ND	0.05	42
N	ND	ND	ND	ND	0.01	21
O	ND	ND	ND	ND	0.01	43
P	ND	ND	ND	ND	0.01	45
Q	ND	ND	ND	ND	0.00	21
R	ND	ND	ND	ND	0.00	19
T	ND	ND	ND	ND	2.48	56
U	ND	ND	ND	ND	1.44	39

[a] ND, not detected by GC/MS. Sample S was unavailable for further testing. All samples except samples T and U were also positive by the EMIT d.a.u.

Table 2. Cross-Reactivity of Selected Drugs with the EMIT d.a.u., EMIT II, and Fluorescence Polarization (FP) Assays for Amphetamine/Methamphetamine

Drug	EMIT d.a.u. (Conc, 10 mg/L)	EMIT II (Conc, 10 mg/L)	FP (Conc, 10 mg/L)	EMIT d.a.u. (Conc, 200 mg/L)	EMIT II (Conc, 200 mg/L)	FP (Conc, 200 mg/L)
Benzphetamine	−	+	−	−	+	−
Chloropromazine	−	−	−	+	−	−
Chloroquine	−	−	−	+	−	−
l-Ephedrine	−	−	−	+	+	−
Mephentermine	−	−	−	−	+	+
N-Acetyl procainamide	−	−	−	+	−	−
Phenmetrazine	−	+	−	+	+	+
Phentermine	+	+	+	+	+	+
Phenylpropanolamine	−	−	−	+	−	+
Procainamide	−	−	−	−	−	−
Pseudoephedrine	−	−	−	−	−	−
Quinicrine	−	−	−	+	−	−
Rantidine	−	−	−	+	−	−
Tyramine	−	−	−	+	−	+

mine in high concentrations is responsible for the unconfirmed positive results.

Four samples (A, B, C, and D) that did not contain amphetamine or methamphetamine were positive by fluorescence polarization with a *d*-amphetamine cutoff of 1.0 mg/L. If the cutoff for fluorescence polarization is lowered to the factory-set value of 0.3 mg/L for *d*-amphetamine, six additional samples not containing amphetamine or methamphetamine would be considered positive by fluorescence polarization. Of these six additional samples, only one was positive by EMIT II and all six were positive by EMIT d.a.u. GC/MS did not identify the compounds responsible for the six additional fluorescence-polarization unconfirmed positives. Therefore, selection of the cutoff concentration for fluorescence polarization affects the number of unconfirmed positives that are caused by analytes other than amphetamine and methamphetamine.

We conclude that the EMIT II shows cross-reactivity for benzphetamine, phenmetrazine, and phentermine as well as *l*-ephedrine and mephentermine (but only at concentrations of 200 mg/L), in addition to reactivity toward *d*- and *l*-amphetamine *d*- and *l*-methamphetamine, MDA, and MDMA. The EMIT II does not detect chloropromazine, chloroquine, *N*-acetylprocainamide, quinicrine, rantidine, and tyramine, which are detected by the EMIT d.a.u.

We thank Lucy Williams of Syva Co. for helpful comments.

References

1. Jaffe JH. Drug addiction and drug abuse. In: Gilman AG, Goodman IS, Gilman L, eds. The pharmacological basis of therapeutics, 6th ed. New York: Macmillan, 1980:535–84.
2. National Institute on Drug Abuse (NIDA). Urine testing for drugs of abuse. NIDA Monog 1986;73:95–7.
3. Litovitz T. In: Haddad L, Winchester JF, eds. Amphetamines in clinical management of poisoning and drug overdose. Philadelphia: WB Saunders, 1983:469–75.
4. Davis JM, Kopin IJ, Lemberger L, Axelrod J. Effects of urinary pH on amphetamine metabolism. Ann N Y Acad Sci 1971;179:493–501.
5. Snyder SH. Catecholamines in the brains as mediators of amphetamine psychosis. Arch Gen Psychiatry 1972;27:169–79.
6. Gorodetzky CW. Detection of drugs of abuse in biological fluids.

In: Martin WR, ed. Drug addiction I. New York: Springer Verlag, 1977: 319–409.
7. Oderta GM, Klein-Schwartz W. Central nervous system stimulants. In: Skoutakis VA, ed. Clinical toxicology of drugs: principles and practices. Philadelphia: Lea and Febiger, 1982:183–99.
8. Bernheim J, Cox JN. Amphetamine overdose in an athlete [Abstract]. Br Med J 1960;ii:590.
9. Zalis EG, Parmley LF. Fatal amphetamine poisoning. Arch Intern Med 1963;112:822–6.
10. Greenwood R, Peachey RS. Acute amphetamine poisoning, an account of 3 cases. Br Med J 1957;i:742–4.
11. Ollerich M. Enzyme immunoassays in clinical chemistry: present status and future trends. J Clin Chem Clin Biochem 1980;18:197–208.

CLIN. CHEM. 39/1, 108–111 (1993)

Phenol and *p*-Cresol Accumulated in Uremic Serum Measured by HPLC with Fluorescence Detection

Toshimitsu Niwa

We developed a simple and sensitive high-performance liquid chromatographic (HPLC) method that uses fluorescence as a detector for quantifying serum phenol and *p*-cresol in uremic patients on hemodialysis. Identification of phenol and *p*-cresol was confirmed by liquid chromatography/mass spectrometry. Because the HPLC method requires only simple extraction by ethyl acetate and does not require further steps such as derivatization, it is simple and rapid compared with gas chromatography or gas chromatography/mass spectrometry. Concentrations of phenol and *p*-cresol in uremic serum were significantly (p <0.01) higher than those in normal serum. Reduction rates of phenol and *p*-cresol by hemodialysis were lower than those of urea and creatinine, suggesting a protein-binding property of phenol and *p*-cresol. This method will be useful for monitoring serum phenols in dialyzed patients as an index of hemodialysis adequacy.

Additional Keyphrases: *chromatography, reversed-phase · hemodialysis · GC/MS compared*

Phenol and *p*-cresol markedly accumulate in the serum of undialyzed and dialyzed uremic patients (*1–6*) and play a role in the development of uremic coma (*7*) and defective platelet aggregation (*8, 9*). Phenol and *p*-cresol are synthesized in the small intestine from tyrosine through 4-hydroxybenzoic acid and 4-hydroxyphenylacetic acid, respectively, by intestinal bacteria (Figure 1) (*10–13*). Aerobic bacteria such as *Enterobacteriaceae* (*Escherichia coli* etc.) and *Streptococcus* tend to produce phenol from tyrosine, whereas anaerobic bacteria such as *Clostridium* and *Bacteroides* produce *p*-cresol (*10–13*). These phenols are absorbed from intestine and normally excreted into urine. In uremia, renal clearance of the phenols is impaired, leading to accumulation of the phenols in the blood.

The serum concentrations of phenol and *p*-cresol in uremic serum have been determined by using colorimetric nitroaniline diazotization (*2*), gas chromatography (GC) (*1*), or gas chromatography/mass spectrometry (GC/MS) (*3, 4*).[1] Plasma phenol in patients treated with phenol in regional nerve blocks was determined by GC/MS (*14*). The colorimetric method is not as specific for phenols as are GC and GC/MS and cannot quantify phenol and *p*-cresol separately. Quantification of phenols by GC or GC/MS is laborious and time consuming because it requires complex extraction and derivatization before analysis. Reversed-phase high-performance liquid chromatography (HPLC), which does not require derivatization, has been used for measuring environmental phenols with electrochemical detection (*15*) and urinary cresols with ultraviolet detection (*16*). However, the HPLC method with electrochemical detection has not been applied to determine serum phenols. When ultraviolet detection was used for determination of serum phenols, the HPLC chromatograms were more complex, with many interfering peaks, and the peaks of phenol and *p*-cresol could not be clearly recognized. We developed a reversed-phase HPLC method for measuring phenol and *p*-cresol in uremic serum. The method is specific and sensitive for phenol and *p*-cresol, uses fluorescence as a detector, and requires only simple extraction before HPLC.

Materials and Methods

Subjects

The study included 17 patients (10 men and 7 women) receiving hemodialysis for 4 h, three times per week. They had been undergoing periodic dialysis for 4.2 (SD 4.0) years (range 0.7–12.6 years). Their mean age was 57.4 (SD 11.1) years (range 34–69 years). Blood samples were taken before and after hemodialysis and allowed to clot. The control subjects were a group of 10 healthy subjects (6 men and 4 women).

Department of Clinical Laboratory, Nagoya University Branch Hospital, 1-1-20, Daiko-minami, Higashi-ku, Nagoya 461, Japan.
Received June 15, 1992; accepted August 21, 1992.

[1] Nonstandard abbreviations: LC/MS, liquid chromatography/mass spectrometry; GC, gas chromatography; GC/MS, gas chromatography/mass spectrometry; APCI, atmospheric-pressure chemical ionization; and SIM, selected ion monitoring.