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Rapid diagnosis of drug intoxication using novel NAGINATA™ gas chromatography/mass spectrometry software

Tomomi Ishida[1], Keiko Kudo[1], Satoko Naka[2], Kenichi Toubou[2], Toshimichi Noguchi[2] and Noriaki Ikeda[1]*

[1]Department of Forensic Pathology and Sciences, Graduate School of Medical Sciences, Kyushu University, Fukuoka 812-8582, Japan
[2]SHINKAWA Electric Co., Ltd., Hiroshima 730-0029, Japan

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In Japan, not only the classical stimulant, methamphetamine, but also a wide variety of illicit drugs and designer drugs are abused by juveniles. It is, however, difficult to screen these drugs in human urine due to the poor availability of high-quality standards. Therefore, it is important to develop a screening method that does not require the use of standard compounds. Furthermore, if we can obtain approximate drug concentrations in biological fluids by the first screening procedure, the subsequent treatment of the patient and forensic diagnosis can be carried out more rapidly and exact quantitative analysis performed more efficiently. We have devised a rapid screening method for the simultaneous semi-quantitative analysis of 30 abused drugs using gas chromatography/mass spectrometry (GC/MS) with a retention time locking technique. Based on this method, an 'abused drugs database' was constructed including retention time (RT), qualifier ion/target ion (QT) percentage and calibration curve (values of slope and intercept) using the novel GC/MS software, NAGINATA™. We compared the analytical results obtained by this method using the constructed database with those from conventional methods in six forensic cases. The number of confirmed drugs and concentrations obtained by the established method was comparable with that obtained by conventional methods. We found a significant improvement in the time for data analysis, and qualitative and quantitative information about each drug was obtained without using standards. Therefore, this new screening procedure using NAGINATA™ has potential for the rapid identification of poisoning and should be useful in clinical and forensic toxicological analyses. Copyright © 2007 John Wiley & Sons, Ltd.

In Japan, not only the classical stimulant, methamphetamine, but also a wide variety of illicit drugs and designer drugs are abused by juveniles[1] and many crimes are committed under the influence of these readily available drugs. These illegal drugs include amphetamines, amphetamine, piperazine, tryptamine and phenethylamine derivatives, opiates and the anesthetic, ketamine. Although there are many methods of determining each drug or some drugs belonging to the same group,[1–11] there are no published procedures for screening all the above drugs simultaneously. Thus, we recently devised a rapid screening method for the simultaneous semi-quantitative analysis of 30 abused drugs including the above in human urine using gas chromatography/mass spectrometry (GC/MS).[12] However, this method requires standard substances for all the target drugs. It is very difficult to obtain these drugs in the form of analytical standards because of strict legal limitations and the high cost of importing drugs for use as standards. Furthermore, analysis of such drugs can be carried out only by laboratories with secure storage space for standard compounds. Therefore, a new method which provides feasible drug screening without using standards is desirable. Furthermore, if drug confirmation and rough estimation of the drug level can be carried out simultaneously by the first screening procedure, the subsequent treatment of the patient and forensic diagnosis can be carried out more efficiently.

A new GC/MS software package has recently been developed by Nishikawa Keisoku Co. Ltd. (Tokyo, Japan), based on the concept of Kadokami *et al.*,[13,14] with several modifications. This software named 'NAGINATA' is designed for system quality control and data analysis as an add-on to the ChemStation software (Agilent Technologies, Santa Clara, CA, USA) used to operate Agilent 6890GC/5973 and 5975MSD mass spectrometers. A system performance check is carried out using a criteria sample mix solution to compensate for instrument-to-instrument and day-to-day condition

**Correspondence to*: N. Ikeda, Department of Forensic Pathology and Sciences, Graduate School of Medical Sciences, Kyushu University, 3-1-1 Maidashi, Higashi-ku, Fukuoka 812-8582, Japan.
E-mail: norii@forensic.med.kyushu-u.ac.jp
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variations before sample measurement. The database consists of the retention time, calibration curve and electron ionization (EI) mass spectrum of each compound. A compound search based on this database is automatically performed after sample measurement. Confirmation (probability of compound inclusion) and tentative quantification values of all compounds are then obtained without running the respective standard compounds. This software database, as commercially available, does not contain drugs of abuse and all information in the database is constructed using standard solutions. For clinical and forensic toxicological purposes, screening methods, including extraction procedures, and a database obtained from the analyses of biological specimens are required.

In the present study, we constructed a database of 30 abused drugs based on our previously reported procedure,[12] and compared the analytical results obtained from the established method using our constructed database with those from conventional methods in six forensic cases. The potential of this screening method using NAGINATA^{TM} was evaluated.

EXPERIMENTAL

Chemicals and reagents

Methamphetamine (MA) hydrochloride was purchased from Dainippon Pharmaceuticals (Osaka, Japan). Phenylpropanolamine (PPA) hydrochloride, 4-bromo-2,5-dimethoxy-β-phenethylamine (2C-B) hydrochloride, mescaline hydrochloride and 5-methoxy-*N*,*N*-dimethyltryptamine (5MeO-DMT) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Methylephedrine (ME), α-methyltryptamine (AMT), *N*-benzylpiperazine (BZP) and 1-(4-methoxyphenyl)piperazine (4MPP) were purchased from Aldrich (Milwaukee, WI, USA). 2,5-Dimethoxy-4-iodo-β-phenethylamine hydrochloride (2C-I), 2,5-dimethoxy-4-ethylthio-β-phenethylamine (2C-T-2) hydrochloride, 2,5-dimethoxy-4-(n)-propylthio-β-phenethylamine (2C-T-7) hydrochloride, 5-methoxy-α-methyltryptamine (5MeO-AMT) hydrochloride and 5-methoxy-*N*,*N*-diisopropyltryptamine (5MeO-DIPT) hydrochloride were synthesized by Chemical Soft R&D Inc. (Kyoto, Japan). 3,4-Methylenedioxyamphetamine (MDA) hydrochloride, 3,4-methylenedioxymethamphetamine (MDMA) hydrochloride, dimethylamphetamine (DMA) hydrochloride, PMA hydrochloride, *p*-methoxymethamphetamine (PMMA) hydrochloride, 4-methylthioamphetamine (4MTA) hydrochloride and psilocin were synthesized in our laboratory using previously published methods.[15–17] Morphine (MOR) hydrochloride was purchased from Sankyo (Tokyo, Japan). Codeine (COD) phosphate and dihydrocodeine (DCO) phosphate were purchased from Takeda Pharmaceuticals (Osaka, Japan). 1-(3-Trifluoromethyphenyl)piperazine (TFMPP) hydrochloride was purchased from Alfa Aesar Avocado Organics (Heysham, UK). Amphetamine (AP) sulfate was a generous gift from the Department of Forensic Medicine,

Table 1. Drug information

Compound	Abbreviation	RT* (min)	Target ion* (*m*/*z*)	Qualifier ion* (*m*/*z*)	Calibration curves*		
					Slope	Intercept	r^2
Dimethylamphetamine	DMA	7.316	72	91	25.01	−4.46	0.992
Methylephedrine	ME	8.654	72	91	23.02	−0.25	0.999
Amphetamine	AP	8.724	86	118	4.38	0.48	0.998
Methamphetamine	MA	9.269	58	100	19.89	2.61	0.997
Phenylpropanolamine	PPA	10.227	86	107	9.79	−2.59	0.977
p-Methoxyamphetamine	PMA	10.325	148	121	11.70	0.36	0.997
Ephedrine	EP	10.595	58	100	5.17	−0.58	0.994
p-Methoxymetamphetamine	PMMA	10.804	58	148	18.06	1.15	0.990
3,4-Methylenedioxyamphetamine	MDA	10.932	162	135	8.94	0.14	0.999
N-Benzylpiperazine	BZP	11.369	91	146	12.15	1.62	0.996
1-(3-Trifluoromethylphenyl)piperazine	TFMPP	11.369	200	188	8.03	0.60	0.996
3,4-Methylenedioxymethamphetamine	MDMA	11.409	58	162	18.00	3.05	0.995
4-Methylthioamphetamine	4MTA	11.414	164	137	6.77	1.73	0.988
N-Methyl-1-(3,4-methylenedioxyphenyl)-2-butanamine	MBDB	11.725	72	176	21.21	2.69	0.993
5-Methoxy-*N*,*N*-dimethyltryptamine	5MeO-DMT	11.812	58	218	18.44	−6.31	0.991
Mescaline		12.072	194	179	6.45	−0.22	0.996
Psilocin		12.513	58	216	1.01	−0.58	0.822
α-Methyltryptamine	AMT	12.543	130	157	4.38	−0.48	0.997
4-Bromo-2,5-dimethoxy-β-phenethylamine	2C-B	12.658	242	229	3.15	−0.09	0.990
1-(3-Chlorophenyl)piperazine	3CPP	12.659	166	238	7.17	0.20	0.999
1-(4-Methoxyphenyl)piperazine	4MPP	12.698	162	234	7.96	−0.27	0.991
Ketamine	KET	12.743	216	152	0.03	0.00	0.926
5-Methoxy-*N*,*N*-diisopropyltryptamine	5MeO-DIPT	13.087	114	160	22.02	−2.54	0.991
2,5-Dimethoxy-4-iodo-β-phenethylamine	2C-I	13.156	290	275	4.16	−0.19	0.997
2,5-Dimethoxy-4-ethylthio-β-phenethylamine	2C-T-2	13.219	224	211	5.70	−0.54	0.992
5-Methoxy-α-methyltryptamine	5MeO-AMT	13.496	160	187	1.25	−0.49	0.978
2,5-Dimethoxy-4-(n)-propylthio-β-phenethylamine	2C-T-7	13.570	238	225	6.71	−1.01	0.980
Dihydrocodeine	DCO	14.142	343	284	2.15	0.06	0.996
Codeine	COD	14.290	341	282	1.58	0.38	0.960
Morphine	MOR	14.888	369	310	0.04	0.00	0.944

* After acetylation (except for DMA, 5MeO-DMT and 5MeO-DIPT).

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Fukuoka University School of Medicine (Fukuoka, Japan). *N*-Methyl-1-(3,4-methylenedioxyphenyl)-2-butanamine (MBDB) was purchased from Cerilliant (Austin, TX, USA). Medazepam hydrochloride was provided by Shionogi & Co. (Osaka, Japan). 1-(3-Chlorophenyl)piperazine (3CPP) hydrochloride and ketamine (KET) hydrochloride were purchased from Wako Pure Chemicals (Osaka, Japan). Ephedrine (EP) hydrochloride was purchased from Junsei Pharmaceuticals (Tokyo, Japan). Table 1 shows the drug names, abbreviations and retention times. A criteria sample mix solution for a system performance check was purchased from Hayashi Pure Chemicals (Osaka, Japan).

Trifluoroacetic acid (TFA), urease from Jack bean (activity, 133 units/mg) and ethyl acetate were purchased from Wako Pure Chemicals. Urease (200 mg) was dissolved in 10 mL distilled water. Acetic anhydride was purchased from Sigma-Aldrich. Pyridine (silylation grade) was purchased from Pierce (Milwaukee, WI, USA). The solid-phase extraction (SPE) column (Focus™) was purchased from Varian Inc. (Lake Forest, CA, USA). The other chemicals were of analytical reagent grade.

Standard solutions

Most drugs (5 mg as free base) were dissolved in methanol and the volume was adjusted to 5 mL, to obtain a concentration of 1000 ng/μL This solution was further diluted in methanol to 100, 10 and 1 ng/μL. PPA, EP, MOR, COD and DCO were dissolved in 0.01 M hydrochloric acid. ME was dissolved in 0.01 M hydrochloric acid containing 0.05% methanol.

Biological samples

Drug-free urine samples obtained from healthy Japanese volunteers and urine samples obtained from autopsy cases were kept at −20°C until analysis.

System performance check of the status in the GC/MS system

The apparatus used was an Agilent 6980 gas chromatograph combined with an Agilent 5973 quadrupole mass spectrometer. NAGINATA™ software (version 1.00.03) was provided by Nishikawa Keisoku Co., Ltd. The GC/MS system performance was evaluated using NAGINATA™ by measuring a criteria sample mix solution. The solution contains 16 criteria compounds (pesticides and environmental substances) and 8 deuterated internal standards (ISs). Table 2 shows the list of criteria compounds and check items corresponding to the status of each part of the GC/MS system. It is known that the peak for each compound is sensitive to any contamination in the system. The following GC/MS conditions were used for the analysis of the criteria sample mix solution: Column HP-5MS (30 m × 0.25 mm i.d., 0.25 μm film thickness) coated with 5% phenyl/95% methylsilicone stationary phase, injection temperature 250°C, oven temperature 70°C (2 min), heated at 25°C/min to 150°C (0 min), at 3°C/min to 200°C (0 min), then at 8°C/min to 280°C (10 min) and EI scan mode. The criteria compounds contain decafluorotriphenylphosphine (DFTPP) and a retention time locking compound (chlorpyrifos-methyl). DFTPP tuning was carried out to obtain a uniform mass spectrum. The retention times of target compounds were fixed using the retention time locking (RTL) method which is known to give retention times with good reproducibility.[18–24]

Construction of 'abused drugs database'

(1) Sample preparation

Urine samples were prepared containing 30 drugs at concentrations of 0.05, 0.1, 0.5, 1.0, 2.5 and 5.0 μg/mL. These samples were extracted and derivatized using our published method[12] with slight modifications. A urine sample (1 mL) was mixed with 1 μL IS solution (1 μg medazepam) in a centrifuge tube (10 mL), and was digested with 200 units of urease at 37°C for 10 min. Then acetone (3 mL) was added

Table 2. Criteria samples for the status of the GC/MS system

			Acceptable ranges			
Check items	Compound	RT (min)	Lower limit of quantification value (%)	Upper limit of quantification value (%)	QT%	Upper limit of tailing factor*
Injector liner	Captafol		>70			
	Isoxathion		>70			
Column(Injector)	2,4-Dichloroaniline		>70			<3
	2,4-Dinitroaniline					<3
	Pentachlorophenol		>50			<3
	Simazine		>70			<1.5
Column(MS)	Fenitrothion		>70			
Ion source	Decafluorotriphenylphosphine (DFTPP)				<15	
Other	2,6-Dichlorophenol		>70	<130		<4
	2,6-Dimethylaniline		>70	<130		<4
	Benzothiazole		>70	<130		<4
	Butyl benzyl phthalate		>70	<130		<1.5
	Diethyl phthalate		>70	<130		<1.5
	Tributyl phosphate		>70	<130		<1.5
	Tris(2-chloroethyl) phosphate		>70	<130		<1.5
Retention time	Chlorpyrifos-methyl	±0.02				

*Tailing factor <1 means leading peak and >1 means tailing peak.

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and the sample was then vortex-mixed for 10 s. The supernatant was transferred to another 10 mL centrifuge tube and evaporated to ca. 0.5 mL under a stream of nitrogen at 60°C to remove acetone. To this solution, distilled water (1 mL) was added, and the mixture was shaken and centrifuged. The supernatant was applied to the Focus™ column previously conditioned sequentially with 1 mL methanol and 1 mL distilled water. The column was rinsed sequentially with 1 mL distilled water and 1 mL 30% acetonitrile (ACN). The analytes were eluted with 1 mL ACN/distilled water/TFA (90:10:0.1, v/v/v). The eluate was then evaporated to dryness under a stream of nitrogen at 60°C. The residue was dissolved in pyridine (50 μL) and acetic anhydride (50 μL) was added to the solution to carry out the acetylation. The mixture was kept at 60°C for 30 min, and the sample was then evaporated to dryness at room temperature. The residue was dissolved in 100 μL ethyl acetate, and a 2 μL aliquot of the solution was injected into the GC/MS system, the performance of which had previously been evaluated.

(2) GC/MS conditions

The GC/MS conditions were as follows: the initial temperature 60°C was held for 2 min, the temperature was then programmed to 300°C at a rate of 20°C/min; this temperature was maintained for 5 min. The injection port and transfer line temperatures were 250 and 280°C, respectively. The carrier gas was helium and the constant pressure mode was used. The retention time was fixed using the retention time locking (RTL) technique. We set the retention time of medazepam (IS) at 13.0 min. The full-scan mode was used.

(3) Registration of each drug on the 'abused drugs database'

One quantifier and one qualifier ion were selected for each drug and a calibration curve was obtained using MSD ChemStation D.02.00.275 by plotting the peak area ratio of the drug to the IS versus the amount of drug. The retention time and the EI mass spectrum of each drug were obtained from the data for the spiked urine sample containing 1 μg/mL drug. The obtained values for 30 drugs – retention times, qualifier ion/target ion (QT) percentages, mass spectra and calibration curve (values of slope and intercept) – were registered as the 'abused drugs database'. Figure 1 shows the scheme of the screening method using the NAGINATA™ software.

(4) Analysis of spiked samples using NAGINATA™

We prepared urine samples (n = 3) spiked with 30 drugs at low (0.1 μg/mL) and high (1.0 μg/mL) concentrations. These spiked urine samples were analyzed using our newly developed method.

(5) Application to actual forensic cases

Urine samples in six forensic cases, where immunoassay screening with Triage™ (Biosite Diagnostics Inc., San Diego,

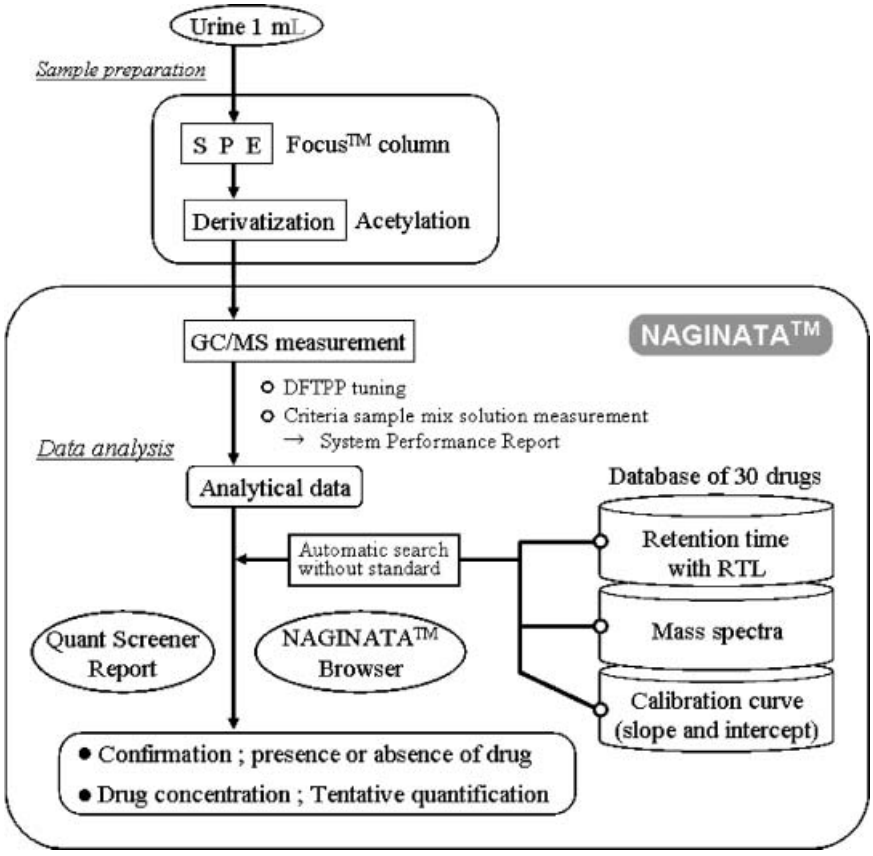

Figure 1. Scheme of the screening method for 30 abused drugs using NAGINATA™ software.

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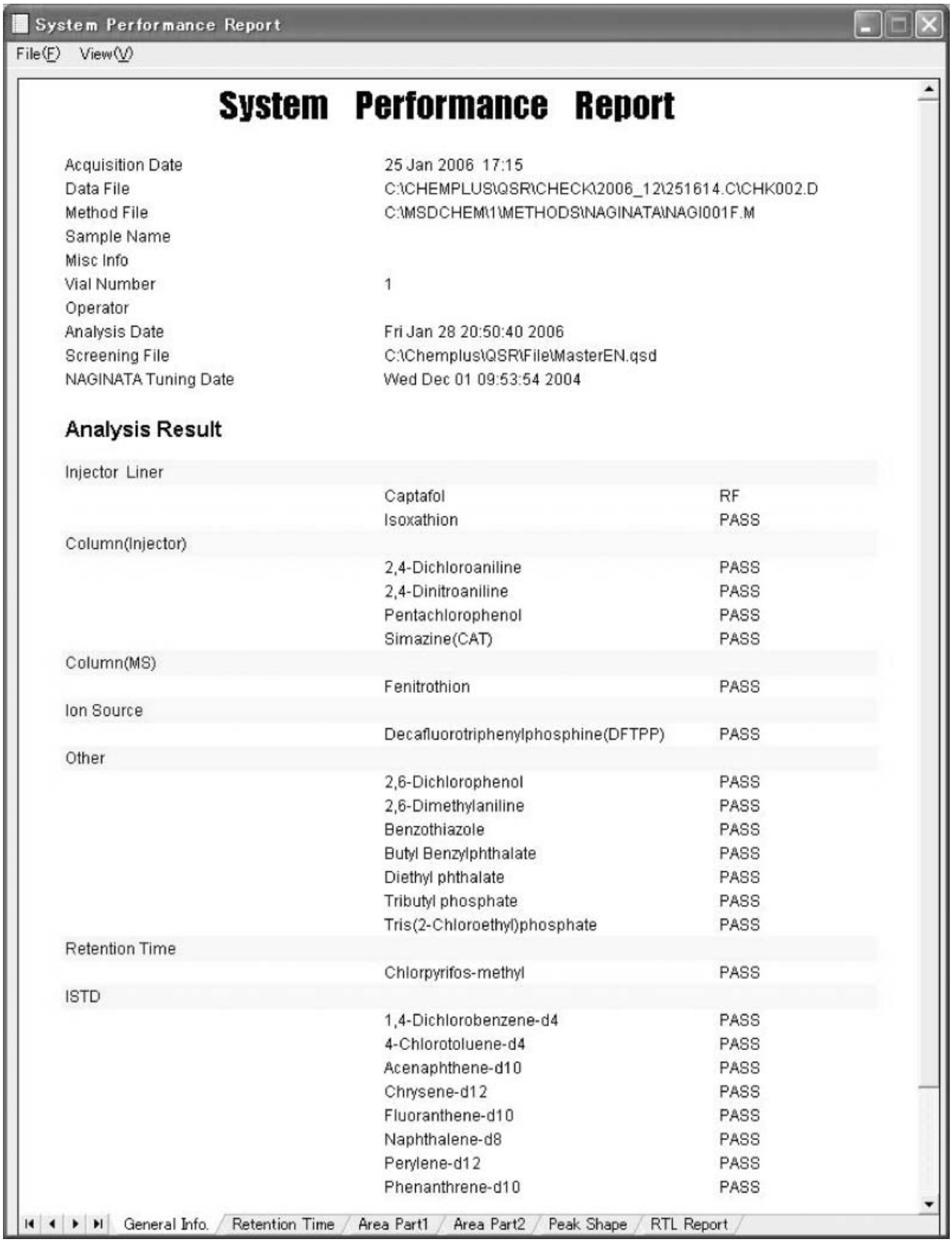
CA, USA) and conventional GC/MS analysis had been already performed, were re-analyzed by our developed method; data analysis was performed using the new 'abused drugs database'.

The results obtained from the established screening method using the database were compared with those previously obtained by the conventional method using standard substances.


System Performance Report

File(F) View(V)

System Performance Report

Acquisition Date	25 Jan 2006 17:15
Data File	C:\CHEMPLUS\QSR\CHECK\2006_12\251614.C\CHK002.D
Method File	C:\MSDCHEM\1\METHODS\NAGINATA\NAGI001F.M
Sample Name	
Misc Info	
Vial Number	1
Operator	
Analysis Date	Fri Jan 28 20:50:40 2006
Screening File	C:\Chemplus\QSR\File\MasterEN.qsd
NAGINATA Tuning Date	Wed Dec 01 09:53:54 2004

Analysis Result

Injector Liner		
	Captafol	RF
	Isoxathion	PASS
Column(Injector)		
	2,4-Dichloroaniline	PASS
	2,4-Dinitroaniline	PASS
	Pentachlorophenol	PASS
	Simazine(CAT)	PASS
Column(MS)		
	Fenitrothion	PASS
Ion Source		
	Decafluorotriphenylphosphine(DFTPP)	PASS
Other		
	2,6-Dichlorophenol	PASS
	2,6-Dimethylaniline	PASS
	Benzothiazole	PASS
	Butyl Benzylphthalate	PASS
	Diethyl phthalate	PASS
	Tributyl phosphate	PASS
	Tris(2-Chloroethyl)phosphate	PASS
Retention Time		
	Chlorpyrifos-methyl	PASS
ISTD		
	1,4-Dichlorobenzene-d4	PASS
	4-Chlorotoluene-d4	PASS
	Acenaphthene-d10	PASS
	Chrysene-d12	PASS
	Fluoranthene-d10	PASS
	Naphthalene-d8	PASS
	Perylene-d12	PASS
	Phenanthrene-d10	PASS

General Info. | Retention Time | Area Part1 | Area Part2 | Peak Shape | RTL Report

Figure 2. System Performance Report. 'PASS' indicates acceptance and 'RF' indicates rejection in each part of the instrument.

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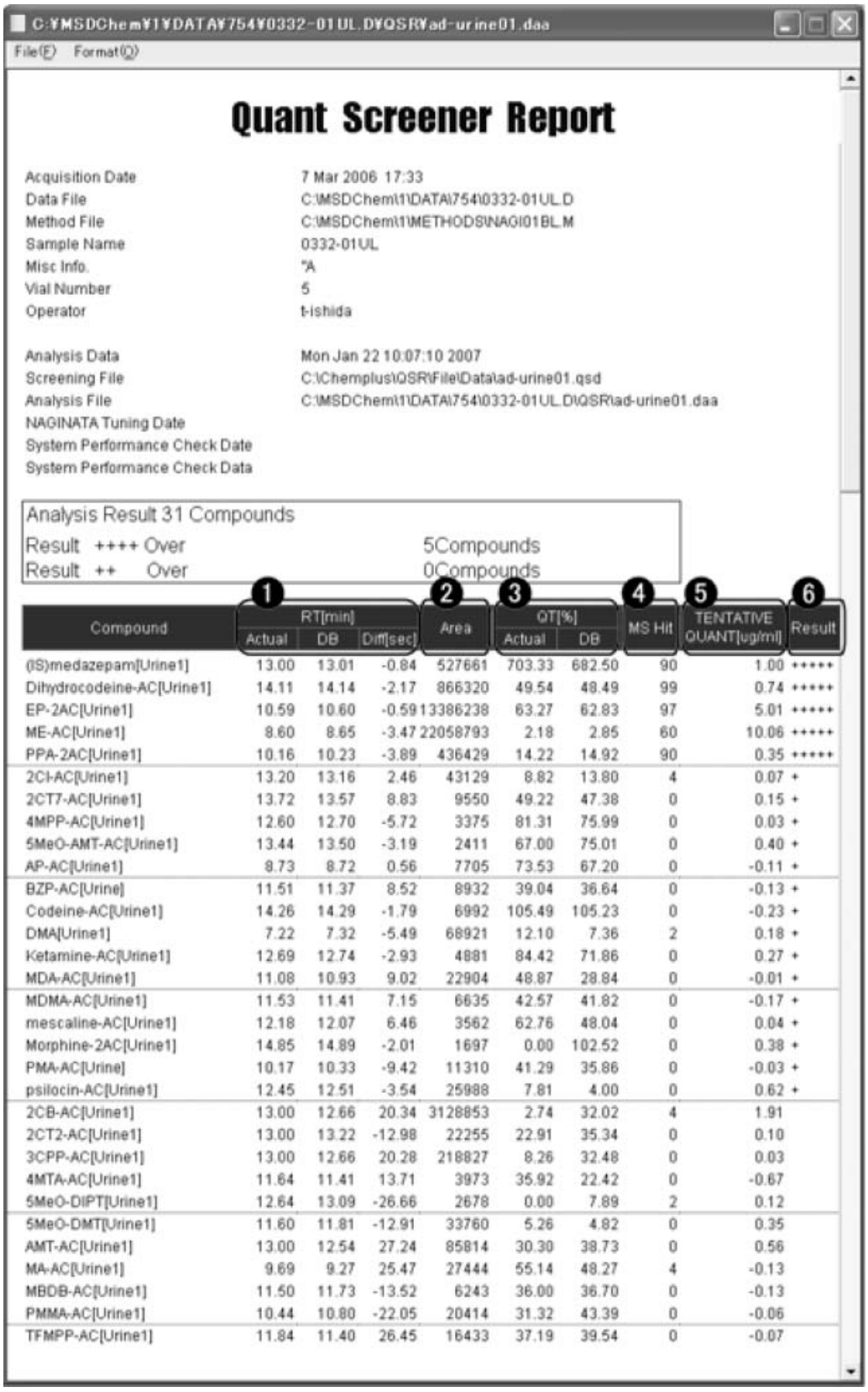

C:¥MSDChem¥1¥DATA¥754¥0332-01UL.D¥QSR¥ad-urine01.daa

File(F) Format(Q)

Quant Screener Report

Acquisition Date	7 Mar 2006 17:33
Data File	C:\MSDChem\1\DATA\754\0332-01UL.D
Method File	C:\MSDChem\1\METHODS\NAGI01BL.M
Sample Name	0332-01UL
Misc Info.	"A
Vial Number	5
Operator	t-ishida
Analysis Data	Mon Jan 22 10:07:10 2007
Screening File	C:\Chemplus\QSR\File\Data\ad-urine01.qsd
Analysis File	C:\MSDChem\1\DATA\754\0332-01UL.D\QSR\ad-urine01.daa
NAGINATA Tuning Date	
System Performance Check Date	
System Performance Check Data	

Analysis Result 31 Compounds

Result ++++ Over 5Compounds

Result ++ Over 0Compounds

Compound	1 RT[min] Actual	RT[min] DB	RT[min] Diff[sec]	2 Area	3 QT[%] Actual	QT[%] DB	4 MS Hit	5 TENTATIVE QUANT[ug/ml]	6 Result
(IS)medazepam[Urine1]	13.00	13.01	-0.84	527661	703.33	682.50	90	1.00	+++++
Dihydrocodeine-AC[Urine1]	14.11	14.14	-2.17	866320	49.54	48.49	99	0.74	+++++
EP-2AC[Urine1]	10.59	10.60	-0.59	13386238	63.27	62.83	97	5.01	+++++
ME-AC[Urine1]	8.60	8.65	-3.47	22058793	2.18	2.85	60	10.06	+++++
PPA-2AC[Urine1]	10.16	10.23	-3.89	436429	14.22	14.92	90	0.35	+++++
2CI-AC[Urine1]	13.20	13.16	2.46	43129	8.82	13.80	4	0.07	+
2CT7-AC[Urine1]	13.72	13.57	8.83	9550	49.22	47.38	0	0.15	+
4MPP-AC[Urine1]	12.60	12.70	-5.72	3375	81.31	75.99	0	0.03	+
5MeO-AMT-AC[Urine1]	13.44	13.50	-3.19	2411	67.00	75.01	0	0.40	+
AP-AC[Urine1]	8.73	8.72	0.56	7705	73.53	67.20	0	-0.11	+
BZP-AC[Urine]	11.51	11.37	8.52	8932	39.04	36.64	0	-0.13	+
Codeine-AC[Urine1]	14.26	14.29	-1.79	6992	105.49	105.23	0	-0.23	+
DMA[Urine1]	7.22	7.32	-5.49	68921	12.10	7.36	2	0.18	+
Ketamine-AC[Urine1]	12.69	12.74	-2.93	4881	84.42	71.86	0	0.27	+
MDA-AC[Urine1]	11.08	10.93	9.02	22904	48.87	28.84	0	-0.01	+
MDMA-AC[Urine1]	11.53	11.41	7.15	6635	42.57	41.82	0	-0.17	+
mescaline-AC[Urine1]	12.18	12.07	6.46	3562	62.76	48.04	0	0.04	+
Morphine-2AC[Urine1]	14.85	14.89	-2.01	1697	0.00	102.52	0	0.38	+
PMA-AC[Urine]	10.17	10.33	-9.42	11310	41.29	35.86	0	-0.03	+
psilocin-AC[Urine1]	12.45	12.51	-3.54	25988	7.81	4.00	0	0.62	+
2CB-AC[Urine1]	13.00	12.66	20.34	3128853	2.74	32.02	4	1.91	
2CT2-AC[Urine1]	13.00	13.22	-12.98	22255	22.91	35.34	0	0.10	
3CPP-AC[Urine1]	13.00	12.66	20.28	218827	8.26	32.48	0	0.03	
4MTA-AC[Urine1]	11.64	11.41	13.71	3973	35.92	22.42	0	-0.67	
5MeO-DIPT[Urine1]	12.64	13.09	-26.66	2678	0.00	7.89	2	0.12	
5MeO-DMT[Urine1]	11.60	11.81	-12.91	33760	5.26	4.82	0	0.35	
AMT-AC[Urine1]	13.00	12.54	27.24	85814	30.30	38.73	0	0.56	
MA-AC[Urine1]	9.69	9.27	25.47	27444	55.14	48.27	4	-0.13	
MBDB-AC[Urine1]	11.50	11.73	-13.52	6243	36.00	36.70	0	-0.13	
PMMA-AC[Urine1]	10.44	10.80	-22.05	20414	31.32	43.39	0	-0.06	
TFMPP-AC[Urine1]	11.84	11.40	26.45	16433	37.19	39.54	0	-0.07	

Figure 3. Quant Screener Report. This report includes the retention time, target ion abundance, QT percentage, quality of matching of mass spectra, and tentative quantification value.

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Table 3. Default setting of confirmation criteria

	Criteria of screening confirmation class				
	+++++	++++	+++	++	+
RT (min)	Within ± 0.2/2	Within ± 0.2/2	Within ± 0.2/2	Within ± 0.2	Within ± 0.2
MS Hit (%)	>70	>50	>50	>50	None
QT (%)	Within ± 20/2	Within ± 20/2	Within ± 20	Within ± 20	None

RESULTS AND DISCUSSION

System performance check of the status in the GC/MS system

A System Performance Report was produced after completion of the analysis of the criteria sample mix solution (Fig. 2). 'PASS' was indicated for parts of the GC/MS system except for the 'Injector Liner, Captafol'. ('RF' is given where the criterion is not met.) The report indicates that all parts of the GC/MS system were in good condition except for the injection liner. A straight liner without wool was originally recommended for the analysis of pesticides and 'PASS' was indicated at every check item with use of this liner (data not shown). The peak intensities for captafol and isoxathion tended to be significantly reduced when using a liner with wool. Since we had to inject extracts from biological samples containing many substances derived from the sample matrix, the use of wool was essential to protect the column. Thus, we arbitrarily set this condition as satisfactory for the analysis of abused drugs. A better criteria sample mix solution can be chosen for the screening of abused drugs in the future.

Qualification and tentative quantification of 30 abused drugs using NAGINATA™

After sample measurement, a Quant Screener Report (QSR), which has drug identification information and tentative quantification values, was displayed (Fig. 3). QSR provided:

1. actual and expected retention time in the database, and their difference;
2. target ion abundance;
3. actual and expected QT percentage;

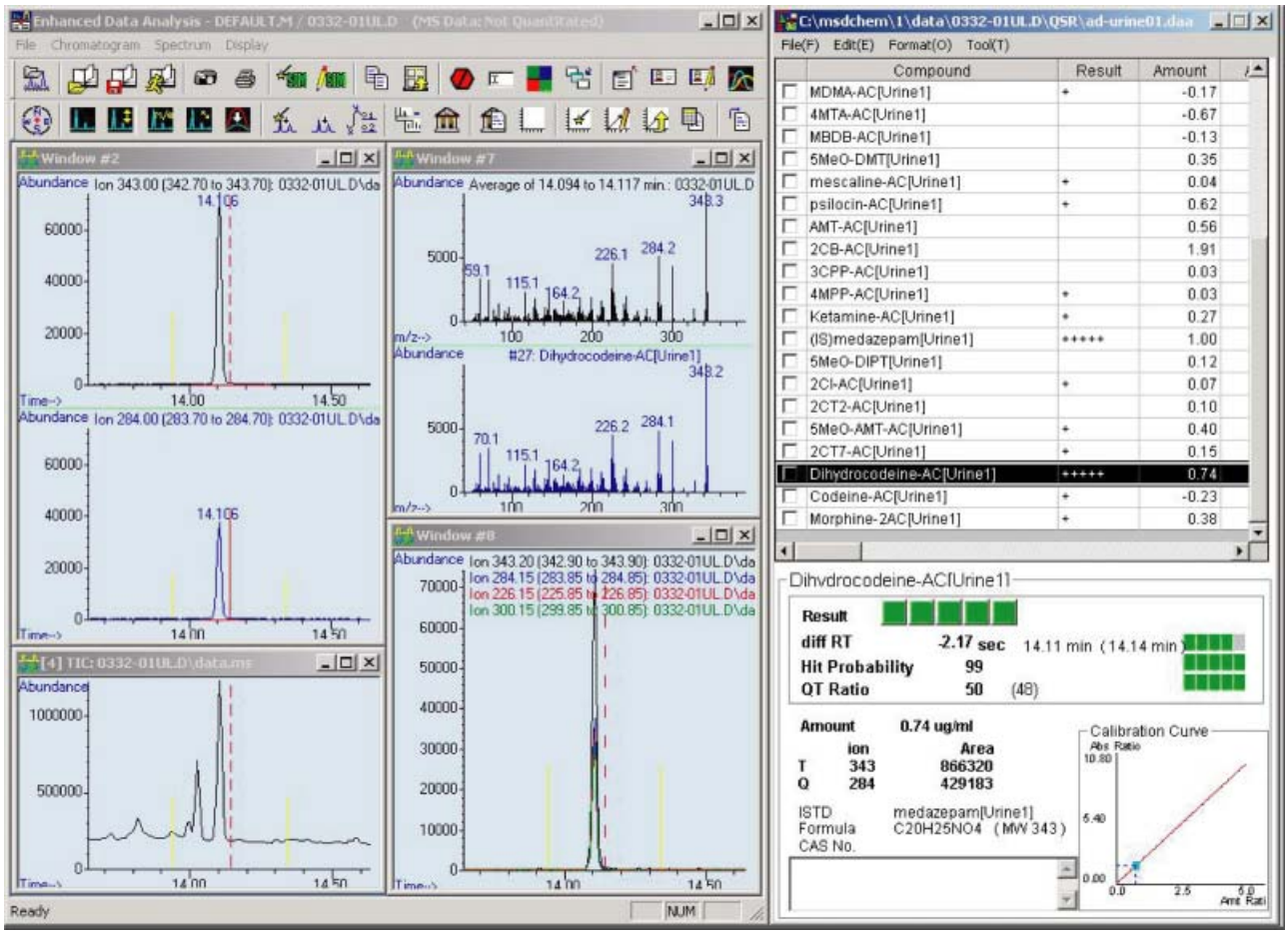

Figure 4. Operation screenshot of 'NAGINATA™ Browser'. The left window includes the ion chromatograms and total ion chromatogram, and the upper middle window (#7) shows the actual and expected mass spectra of the peak and the lower middle window (#8) shows chromatograms of the top four ions with highest abundance.

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4. the agreement between the actual and expected mass spectra calculated with a Probability-Based-Matching algorithm;[25]
5. tentative quantification value; and
6. screening confirmation class. The confirmation class was divided into six levels from +++++ marks to no mark. This mark indicates the probability of the drug being present based on the criteria, as shown in Table 3. By looking at the QSR screen, the presence of EP, ME, PPA and DCO in the sample was quickly confirmed.

Figure 4 shows the 'NAGINATA™ browser' for the analysis of each drug. When we click the name of the drug in the upper right window, all data corresponding to this drug are shown in the other windows of this screen. The chromatograms of the target and qualifier ions and the total ion chromatogram are presented in the upper (#2) and lower left windows, respectively. The upper middle window (#7) shows the actual and expected mass spectra of the peak and the lower middle window (#8) contains chromatograms of the four ions with the highest abundances. The lower right window contains the qualification and quantification results for the drug.

Table 4 shows the results of the screening for urine samples spiked with 30 abused drugs at concentrations of 0.1 μg/mL and 1.0 μg/mL and the lower limit of detection of each drug. An automatic search showed the presence of drugs with more than +++ mark in 26 drugs at a concentration of 1.0 μg/mL and in 16 drugs at a concentration of 0.1 μg/mL. The drug confirmation rate tended to become lower at low concentrations because the mass spectra were of poorer quality. However, the presence of drugs was manually confirmed by using the NAGINATA™ browser. The quantification values at a concentration of 1.0 μg/mL were less variable than at 0.1 μg/mL. The limit of detection for each drug, at a signal-to-noise ratio of 3, ranged from 0.01 to 0.06 μg/mL.

Comparison of two methods, NAGINATA™ screening and the conventional method

Table 5 shows the results obtained by the conventional method and NAGINATA™ screening in six forensic cases where Triage™ screening had given positive results (amphetamines (AMP) in cases 1–5, opiates (OPI) in case 6). The results of the Triage™ screening agreed with those of the NAGINATA™ screening in all cases and the tentative concentrations of MA (cases 2–5), EP (cases 1, 2 and 6), AP (case 3), ME (case 6) and DCO (case 6) obtained from the 'abused drugs database' were within 50 to 150% of the values obtained by the conventional method. In cases 2, 4 and 5, the

Table 4. Results of NAGINATA™ screening for urine samples spiked with 30 abused drugs (n = 3) and lower limit of detection

		0.1 μg/mL		1.0 μg/mL		
Drug name	RT (min)	Drug confirmation	Tentative quantification value (μg/mL)	Drug confirmation	Tentative quantification value (μg/mL)	Limit of detection (μg/mL)
DMA	7.316	+	0.20 ± 0.03	++	1.01 ± 0.09	0.04
ME	8.654	+++++	0.15 ± 0.08	+++++	1.05 ± 0.06	0.01
AP	8.724	+	0.01 ± 0.08	+++++	0.99 ± 0.06	0.05
MA	9.269	+	0.02 ± 0.09	+++++	1.01 ± 0.10	0.01
PPA	10.227	+++++	0.34 ± 0.05	+++++	1.21 ± 0.05	0.01
PMA	10.325	+	0.07 ± 0.05	+++++	0.98 ± 0.05	0.01
EP	10.595	+++	0.52 ± 0.30	+++++	1.04 ± 0.10	0.01
PMMA	10.804	+++++	0.05 ± 0.07	+++++	1.04 ± 0.12	0.01
MDA	10.932	+++	0.08 ± 0.06	+++++	1.00 ± 0.03	0.01
BZP	11.369	+++++	0.06 ± 0.20	+++++	1.00 ± 0.07	0.01
TFMPP	11.369	+++	0.11 ± 0.15	+++++	1.00 ± 0.12	0.01
MDMA	11.409	+	−0.10 ± 0.03	+++++	1.08 ± 0.11	0.01
4MTA	11.414	+++++	−0.45 ± 0.19	+++++	1.29 ± 0.10	0.01
MBDB	11.725	+++++	−0.02 ± 0.08	+++++	1.11 ± 0.12	0.01
5MeO-DMT	11.812	+++++	0.52 ± 0.09	+++++	0.86 ± 0.11	0.03
Mescaline	12.072	+	0.38 ± 0.28	+++++	0.96 ± 0.09	0.01
Psilocin	12.513	+	0.46 ± 0.11	+++++	1.10 ± 0.50	0.05
AMT	12.543	+++++	0.73 ± 0.15	+	1.15 ± 0.11	0.03
2C-B	12.658	+++++	0.30 ± 0.16	+++++	1.02 ± 0.05	0.02
3CPP	12.659	+	0.09 ± 0.08	+++++	0.94 ± 0.10	0.03
4MPP	12.698	+	0.07 ± 0.02	+++++	0.96 ± 0.11	0.05
KET	12.743	+		+	0.99 ± 0.45	0.01
5MeO-DIPT	13.087	+	0.21 ± 0.04	+++++	0.96 ± 0.05	0.01
2C-I	13.156	+++++	0.27 ± 0.14	+++++	0.97 ± 0.08	0.01
2C-T-2	13.219	+++++	0.35 ± 0.19	+++++	0.91 ± 0.04	0.01
5MeO-AMT	13.496	+	0.68 ± 0.28	+	0.86 ± 0.11	0.06
2C-T-7	13.570	+++	0.40 ± 0.17	+++++	0.95 ± 0.08	0.01
DCO	14.142	+++	0.15 ± 0.11	+++++	0.83 ± 0.19	0.02
COD	14.290	+	0.03 ± 0.16	+++++	0.77 ± 0.44	0.02
MOR	14.888	+	0.40 ± 0.08	+++++	0.74 ± 0.32	0.02
		Average	0.21 ± 0.08	Average	0.99 ± 0.13	

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Table 5. Comparison of results from conventional analytical method with those from NAGINATA™ screening

		Conventional analytical method by GC/MS		NAGINATA™ screening	
Case no.	Triage™	Confirmation	Concentration (μg/mL)	Confirmation	Concentration (μg/mL)
1	AMP (+)	EP	17.06	EP +++++	22.59
				ME +++++	0.82
2	AMP (+)	MA AP	168 7.25	MA +++++	123.34
				EP +++++	0.75
3	AMP (+)	MA AP	23.3 0.51	MA +++++	32.56
				AP +++++	0.71
4	AMP (+)	MA AP	5.22 0.34	MA +++++	5.32
5	AMP (+)	MA AP	7.99 2.08	MA +++++	9.86
				PPA +++++	0.38
6	OPI (+)	EP ME DCO	2.45 8.31 0.83	EP +++++	3.61
				ME +++++	9.25
				DCO +++++	0.86

presence of AP was not automatically detected using the database search. As the presence of AP was confirmed using the NAGINATA™ browser in every case, NAGINATA™ screening was performed again, after lowering the criterion in Table 3 as follows: more than 50% of MS Hit to be +++++ and ++++, and 25–50% to be +++. As a result, the confirmation class of AP in case 4 was changed from no mark to ++++ with a concentration of 0.36 μg/mL and that in case 5 was changed to ++ with a concentration of 3.08 μg/mL. In case 2, the confirmation class of AP was still indicated as no mark probably due to the shift in retention time caused by the extremely high concentration of MA. Therefore, the degree of peak tailing and/or peak shape may affect the automatic search for compounds using the database. Thus, the automatic search condition should be further investigated.

In cases 2 and 5, EP (case 2) and PPA (case 5) were identified using NAGINATA™ screening in spite of these compounds not being identified by the conventional analytical method[26] developed for the analyses of MA, AP and their metabolites. In this method, heptafluorobutyroyl (HFB) derivatization was performed and the selected ion monitoring (SIM) mode was used following the Triage™ positive result for AMP. Therefore, it was possible to overlook some drugs when the specified method was used for target compounds only.

In a typical toxicological analysis, immunoassay screening is first performed and GC/MS screening is then carried out using commercially available mass spectral libraries, such as NIST and PMW. The NIST Mass Spectral Library (2002 version) contains data files of 174 948 spectra and 146 198 chemical structures in Agilent ChemStation format. PMW (Pfleger/Maurer/Weber) contains 6350 spectra in Agilent ChemStation format. These libraries do not contain retention times; therefore, the process of finding compounds is often based on the experience of the toxicologist. Once we find possible compounds, confirmation and quantification using a calibration curve have to be carried out. These analyses not only take several days but also need standard substances. In NAGINATA™ screening, we achieved drug confirmation and a rough estimation of the drug level rapidly without using standards, and in the data analysis step we could clearly and easily view the chromatogram and mass spectrum of each drug using the NAGINATA™ browser. Although urine drug concentration cannot always be directly connected to the level of poisoning, drug confirmation and approximate concentration in the first screening procedure should be helpful for the treatment of the patient and forensic diagnosis. We think that a similar approach can be used for blood analysis. Therefore, NAGINATA™ software screening should be useful in clinical as well as in forensic cases.

Deconvolution Reporting Software (DRS; Agilent Technologies)[24,27] is an applications package that can be used in an automatic screening procedure. This software detects substances based on the deconvolution of the target peak from the overlapping matrix peaks. The software finds possible compounds in the 'cleaned spectra' by using a prepared database that contains the retention times and mass spectra of pesticides and environmental compounds. The principle of this software is similar to that of NAGINATA™. However, DRS does not contain a function for a system performance check and quantification is carried out using ether a one-point calibration curve or the ChemStation database. Therefore, our screening method using NAGINATA™ software was considered to be superior in both qualification and quantification analysis to the screening method using DRS.

CONCLUSIONS

In Japan, multi-analyte procedures without using standard substances are needed because of the reasons described above. We evaluated the potential of our screening method using NAGINATA™ software and found that the instrument status was constant for all the Agilent instruments tested by measuring a criteria sample mix solution before sample measurement, that exact drug confirmation and tentative quantification could be carried out without using standard substances, and that data analysis after sample measurement was simple and the time for data analyses was significantly shortened. Therefore, our new screening procedure using NAGINATA™ software allows very rapid investigation of poisoning and should be useful in clinical and forensic toxicological cases.

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