TECHNICAL NOTE

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Use of REMEDi HS in Emergency Toxicology for a Rapid Estimate of Drug Concentrations in Urine, Serum, and Gastric Samples

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ABSTRACT: The REMEDi HS is a broad spectrum drug identification system, designed for emergency toxicology screening and forensic applications. The total analysis time is about 20 min. The current library has 555 drugs and metabolites. The system has a software routine that uses an internal standard (IS) to perform quantitative analysis for target compounds when calibrators are available; further, response factors (RF) are supplied for a rapid estimate of drug concentrations when calibrators are unavailable. In the present study, the concentrations of six drugs (bromisovalum, ephedrine, hydroxyzine, diphenhydramine, ranitidine, and lidocaine) and a metabolite of lidocaine (glycinexylidide) were determined using both methods. The slopes of the regression lines between the rapid estimate method and the IS method were generally within 20% of unity, in agreement with the manufacturer's claim. Semiquantitative estimates based on RF also showed good agreement with results obtained using multipoint calibration. These estimates were sufficient for clinical differentiation of routine and toxic levels. Our study demonstrated that the REMEDi HS is particularly useful for a rapid estimate of drug concentrations in the samples from emergency cases when calibrators are not readily available. Our study also showed that this system can be used for the therapeutic monitoring of ranitidine, bromisovalum, lidocaine, and diphenhydramine.

KEYWORDS: forensic science, forensic toxicology, REMEDi HS, emergency medicine, bromisovalum, ephedrine, hydroxyzine, diphenhydramine, ranitidine, lidocaine, glycinexylidide

The quantitative analysis of drugs and poisons identified by rapid screening is very useful for the treatment of intoxication. Particularly in emergency medicine, quantitative analysis should be performed as rapidly as possible so that the appropriate treatment can be selected. There are, however, few instruments that are suitable for both rapid qualitative screening and quantitative analysis. REMEDi HS (Bio-Rad Laboratories, Hercules, CA, US), a broad spectrum drug identification system was evaluated for this type of application.

REMEDi HS, which consists of a high performance liquid chromatography (HPLC) system coupled with multiwavelength ultraviolet detection, can screen 555 drugs and metabolites. The performance of this system for drug screening was reported previously (1–8). The REMEDi HS has a software routine for quantitative analysis using an IS when calibrators are available. Because it is impractical to stock several hundred drug standards and to predict fully screening results for emergency samples, the system also provides a list of response factors (RF) for most drugs in the library so that a rapid estimate of drug concentrations can be obtained immediately after the screening.

In this study, we have evaluated the use of RF for rapid estimate of drug concentrations. In our experience, bromisovalum, ephedrine, hydroxyzine, diphenhydramine, ranitidine, and lidocaine have been difficult to screen by immunoassay, and we have used the REMEDi HS to detect these drugs in serum, urine, and gastric matrices; the rapid estimates were correlated with the internal standard method.

Materials and Methods

Specimens

Table 1 lists the specimens evaluated that contained the six different drugs (bromisovalum, ephedrine, hydroxyzine, diphenhydramine, ranitidine, and lidocaine) and one lidocaine metabolite. Specimens were obtained from emergency medical cases, autopsy cases, human volunteers, and animal studies. The specimens were stored at 4°C and analyzed within 48 h after collection.

Written consent was obtained from all volunteers before experimental administration, and the animal experiments were performed according to the Guidelines for the Care and Use of Laboratory Animals in Takara-machi Campus of Kanazawa University.

Reagents and Standards

All reagents used for REMEDi HS were purchased from Nippon Bio-Rad Laboratories (Tokyo, Japan). Bromisovalum and ephedrine were purchased from Nippon Shinyaku (Kyoto, Japan) and Dai-Nippon Pharmaceutical (Osaka, Japan), respectively. The other five drugs were kindly donated by the following pharmaceutical companies: hydroxyzine, Pfizer Japan (Tokyo, Japan); ranitidine,

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Sankyo (Tokyo, Japan); diphenhydramine, Kowa (Nagoya, Japan); lidocaine and glycinexylidide (a metabolite of lidocaine), Astra Japan (Osaka, Japan). Stock solutions of drug standards were prepared in ethanol at a concentration of 1 mg/mL.

REMEDi HS System

The REMEDi HS system is based upon multicolumn HPLC with UV spectral scanning. It consists of two polymeric cartridges for on-line sample preparation and two analytical cartridges for separation. One analytical cartridge contains a reversed phase packing that separates neutral and weakly basic drugs; the other analytical cartridge contains unmodified silica that separates basic drugs. Acidic drugs are removed by the second cleaning cartridge. The detailed configuration and mobile phase of REMEDi HS were reported previously (9,10). Sample preparation procedure for serum, urine, and gastric samples are shown in Fig. 1. One milliliter of sample was mixed with 0.2 mL IS combination solution, consisting of *N*-ethylnordiazepam (IS 1; 2 μg/mL in final solution) and chlorpheniramine (IS 2; 3 μg/mL in final solution).

The software (Version 4.13) in use contained 555 drugs and metabolites. The identifications of drugs and metabolites are made by evaluation of their relative retention indices and UV spectral parameters, including matching fitness throughout the measured UV range (205 to 300 nm).

Rapid Estimate of Drug Concentrations

The REMEDi HS system provides response factors in the on-line drug library. The rapid estimate of drug concentration is calculated by the operator according to the following equation:

$$\text{Drug concentration, } \mu\text{g/mL} = \text{Response Factor} \times \{\text{Peak height of the drug in chromatogram}\} \times 10^{-3}$$

The response factor is determined by the manufacturer, based upon the average ratio of drug concentration against the peak

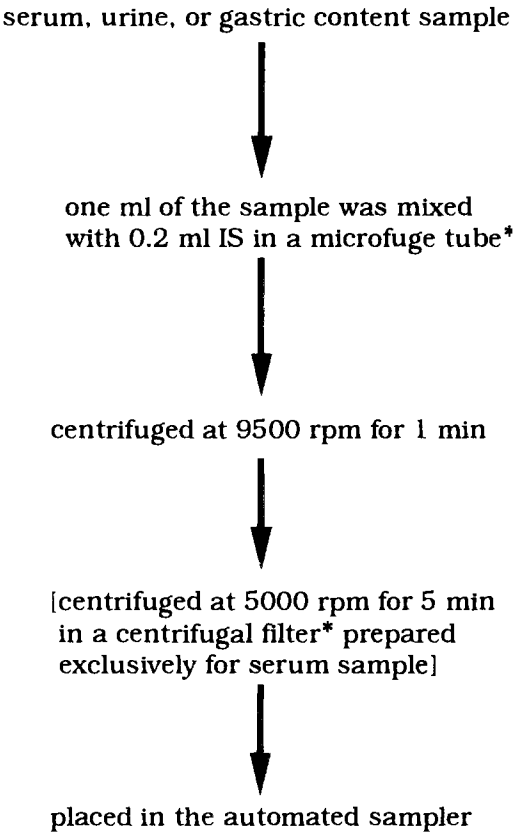

FIG. 1—*Sample preparation procedure for REMEDi HS system. *-microfuge tube and centrifugal filter were prepared for exclusive use with REMEDi HS.*

TABLE 1—*Profile of specimens.*

Drug Name	RF	Pharmacological Action	Specimens Tested	Source*
Bromisovalum	0.0109	Hypotonic-sedative	Serum (n = 6) Urine (n = 5) GC† (n = 2)	63-year-old male (E) 26-year-old female (A)
Ephedrine	0.0130	β 2-stimulant	Urine (n = 74)	Exp; 6 male volunteers‡ (aged 25 to 40 years)
Hydroxyzine	0.0044	Antianxiety agent	Urine (n = 6) GC (n = 7)	26-year-old male (E) 26-year-old female (A)
Diphenhydramine	0.0055	Antihistamine	Serum (n = 71) Urine (n = 83)	Exp; 17 male volunteers‡ (aged 18 to 27 years)
Ranitidine	0.0188	H_2-blocker	Urine (n = 5)	63-year-old male (E)
Lidocaine	0.0042	Local anesthetic, antiarrythmic	Serum (n = 3) Urine (n = 6)	Exp; 2 rabbits§ (weight; ca. 2 kg)
Glycinexylidide	0.0043	Metabolite of lidocaine	Serum (n = 5) Urine (n = 5)	Exp; 2 rabbits§ (weight; ca. 2 kg)

*where
n = the number of samples analyzed
E = emergency medical case.
A = autopsy case.
Exp = experimental administration.
†Gastric content.
‡These drugs were orally administered to male volunteers.
§Lidocaine was subcutaneously injected into two Japanese white rabbits.

TABLE 2—*Concentrations of spiked samples.*

Drug Name	Concentrations, μg/mL
Bromisovalum	1.0, 3.0, 7.0, 10.0
Ephedrine	0.10, 0.30, 0.50, 1.0, 3.0, 3.5
Hydroxyzine	0.08, 0.40, 1.5, 2.5
Diphenhydramine	0.04, 0.09, 0.40, 2.0, 3.0
Ranitidine	0.03, 0.30, 3.0, 5.0
Lidocaine	0.10, 0.15, 0.50, 2.0, 2.5
Glycinexylidide	0.08, 0.15, 0.40, 1.5, 2.0

height across the linearity range (0.0 to 0.8 A.U.), using drug supplemented urine or serum standards.

Internal Standard Method

The peak height of each substance relative to chlorpheniramine (IS2) was used to calculate the concentration. In addition, for the evaluation of linearity, various concentrations of the seven drug standards were prepared in serum, urine, and aqueous matrices. They were analyzed by the quantitative software routine of REMEDi HS system, and their peak heights were also used to prepare calibration curves. The concentrations of these spiked samples were listed in Table 2. The detection limits of these seven analytes were obtained from the lowest concentrations of the spiked samples which were identified by REMEDi HS. For example, ranitidine at 0.03 μg/mL gave a detectable peak but was not identified by the spectral comparison; the detection limit was established at 0.3 μg/mL.

Results and Discussion

Retention Times and Relative Retention Times

Relative retention times (RRT) were used to monitor the elution of each drug and metabolite, because they can compensate for the small variation of retention time during the lifetime of the cartridges. The RRT is obtained by ratio of the adjusted RT of the analyte against the IS, and is more reproducible than RT. The retention relative to IS2 showed a standard deviation of less than 0.01 (about 0.1 min) for most of the drugs evaluated. The RT adjustment, 82 s, corresponding to the time required for an unretained species to pass through the last three cartridges.

Simultaneous Detection of Parent Compound and Metabolites

One of the features of REMEDi HS is that both the parent compound and its metabolites can be detected in the same run. The simultaneous detection of the drug metabolites further confirms the intake of the corresponding parent drugs. In this study, we identified a metabolite of hydroxyzine, *N*-desmethyldiphenhydramine and another metabolite of diphenhydramine, two different metabolites of ranitidine, and monoethylglycinexylidide (another metabolite of lidocaine).

Linearity

The calibration curve of each drug was linear within the tested ranges (Table 2). The highest concentrations were from 10 to 75 times of the lowest concentration.

Correlation of Rapid Estimate Method with IS Method

Table 3 summarizes the correlation of the drug concentrations obtained from rapid estimate method and IS method. Figure 2 shows the relationship between the measured concentrations by these two methods of all seven analytes in serum, urine, and gastric specimens.

Bromisovalum—The bromisovalum concentrations obtained in all specimens (serum, urine, and gastric contents) were almost identical for both methods. The lower detection limit of bromisovalum by REMEDi HS was 1 μg/mL in serum. A previous report indicated that the toxic level of this drug in blood was 24.9 μg/mL (11). Therefore, the rapid estimate method can provide a relatively accurate information of quantitation for bromisovalum in serum and urine samples. Only two gastric specimens were analyzed in this study.

Ephedrine—For ephedrine, the concentrations determined by the rapid estimate method were about 2.2 times as large as those

TABLE 3—*Regression lines and correlation coefficients between Rapid Estimate Method and Internal Standard Method.*

Drug Name	Specimen	Regression Line*	Correlation Coefficient
Bromisovalum	Serum	Y = 1.072X − 0.481	0.973(n = 6)
Bromisovalum	Urine	Y = 1.026X − 0.291	0.960(n = 5)
Bromisovalum	GC†	Y = 0.858X − 0.117	NC‡(n = 2)
Ephedrine	Urine	Y = 2.173X − 0.087	0.983(n = 74)
Hydroxyzine	Urine	Y = 1.120X − 0.071	0.999(n = 6)
Hydroxyzine	GC	Y = 1.068X − 0.047	1.000(n = 7)
Diphenhydramine	Serum	Y = 1.047X + 0.013	0.992(n = 71)
Diphenhydramine	Urine	Y = 1.151X − 0.014	0.973(n = 83)
Ranitidine	Urine	Y = 0.940X + 0.099	0.975(n = 5)
Lidocaine	Serum	Y = 0.856X − 0.012	NC(n = 3)
Lidocaine	Urine	Y = 1.129X − 0.071	0.997(n = 6)
Glycinexylidide	Serum	Y = 0.850X + 0.050	0.996(n = 5)
Glycinexylidide	Urine	Y = 1.152X − 0.061	1.000(n = 5)

*where
Y = concentration measured by Rapid Estimate Method.
X = concentration measured by Internal Standard Method.
†Gastric content.
‡Not calculated.

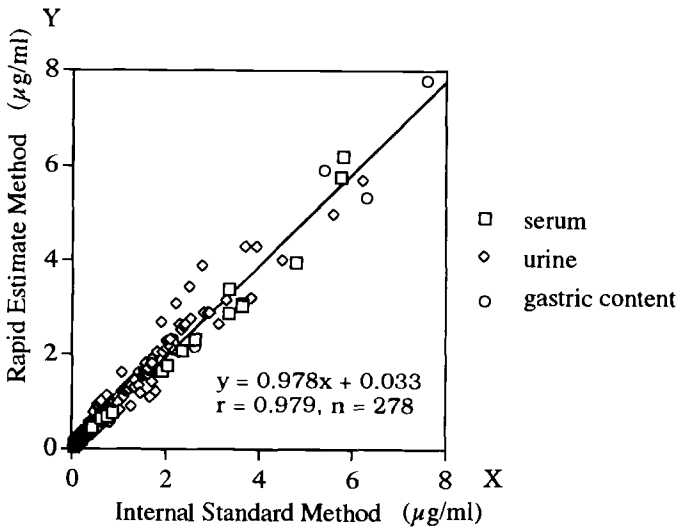

FIG. 2—*Relationship between concentrations of all seven analytes measured by Rapid Estimate Method and Internal Standard Method.*

determined by IS method. However, this RF value of ephedrine is still adequate to distinguish between routine and toxic levels. It has been reported that the single dose peak level in serum is from 0.04 to 0.14 μg/mL, and the therapeutic range is 0.04 to 0.08 μg/mL (12, p. 585). The toxic level in serum was reported to be above 20 μg/mL (13). When we adjusted the RF of ephedrine to 0.0058, the values from both methods were identical.

The detection limit of ephedrine in serum and urine specimens was determined to be 0.1 μg/mL. In the present study, ephedrine was not detected in the serum specimens because the concentration was probably below the detection limit (14). Considerable levels of intact ephedrine are regularly identified in the urine of patients taking ephedrine medications. Intoxication information should be obtained from patient serum samples.

Hydroxyzine—The hydroxyzine concentrations in urine and gastric specimens showed a good correlation by both methods, and the values obtained from both methods were almost identical. Therefore, the rapid estimate method is useful for obtaining the quantitation information of hydroxyzine in urine and gastric specimens.

The detection limit of hydroxyzine was determined to be 0.08 μg/mL in both urine and serum specimens. The peak level of hydroxyzine in plasma, following therapeutic dose ingestion, is from 0.074 to 0.083 μg/mL (12, p. 674). Considering a reported lethal concentration of 39 μg/mL in blood (15), the REMEDi HS seems useful for detection of hydroxyzine intoxication.

Diphenhydramine—The concentrations obtained from rapid estimate method were very similar to those determined by IS method.

The lower detection limit of diphenhydramine in serum and urine specimens was determined to be 0.04 μg/mL, and it is apparently low enough to determine diphenhydramine intoxication

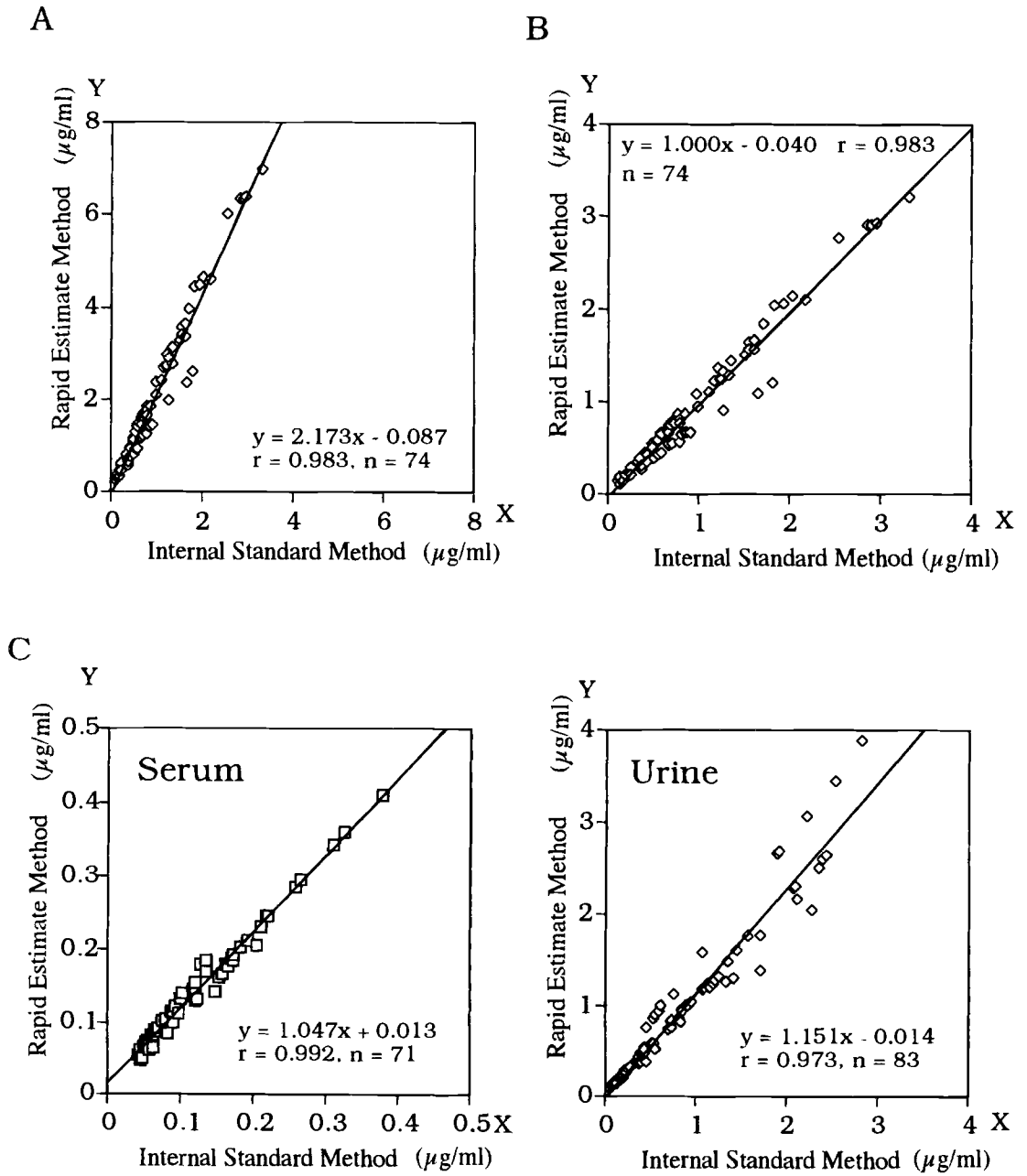

FIG. 3—*Regression lines between Rapid Estimate Method and Internal Standard Method: (A) ephedrine (urine, current RF); (B) ephedrine (urine, adjusted RF); (C) diphenhydramine (serum); diphenhydramine (urine).*

(16–18). In plasma, the concentration is usually in the range of 0.1 to 1.0 μg/mL; a toxic effect could be produced when the plasma concentration is greater than 1 μg/mL (12, p. 558). REMEDi HS would also be useful for therapeutic drug monitoring (TDM) of diphenhydramine.

Ranitidine—The concentrations in urine specimens were similar for both rapid estimate method and IS method. The lower detection limit of ranitidine in the serum and urine specimens was about 0.3 μg/mL. The plasma concentrations were reported to be from 0.31 to 0.82 μg/mL (12, p. 957). REMEDi would be useful for TDM of ranitidine.

Lidocaine and its Metabolite, Glycinexylidide (GX)—The concentration values obtained from both methods were very similar for lidocaine and GX in serum and urine specimens.

Lidocaine is a local anesthetic and antiarrhythmic drug. The detection limit for lidocaine in serum and urine specimens was determined to be 0.1 μg/mL, and it is sufficient for TDM as well as for the diagnosis of intoxication when considering its therapeutic and toxic levels (12, p. 706, 19, 20). The therapeutic level is from 2.0 to 5.0 μg/mL; toxic level in plasma is greater than 6.0 μg/mL; the fatalities level with blood concentrations is greater than 14 μg/mL (21). TDM of lidocaine is commonly carried out in this region.

Broad Spectrum Drug Identification and Rapid Estimation of Quantitation

There are many methods available for drug screening (qualification) and quantitation. However, in the clinical laboratory setting, rapid analysis time and ease of usage are primary considerations, in addition to the ability to identify a broad spectrum of drugs without changing analysis conditions. In gas chromatographic (GC) analysis, off-line sample preparation may be time-consuming and thermally labile compounds cannot be detected. The identification of a drug in GC is based on retention index only. The GC-MS method is usually used only for confirmation, and it shares the same limitations of GC for drugs such as ranitidine.

The advantage of HPLC includes the ability to analyze thermally labile and nonvolatile compounds. In an HPLC system such as REMEDi HS, both retention indices and full UV spectrum comparison are used for the identification of drugs and metabolites. It can screen over 500 drugs using the current library in about 20 min, and the user can also add new drugs into his or her own library. A minimal sample pretreatment is required, and an automatic on-line sample preparation and cleaning up process is incorporated (9,10).

In our opinion, a rapid quantification of drug concentration following screening is very important in both emergency medicine and forensic practices. We have previously evaluated the use of REMEDi HS with calibrators as a tool for quantitative analysis (2). In emergency toxicology, a required drug standard for quantitative analysis might not be available and quantitative information can not be obtained so immediately in most cases. Unfortunately, access of drug standards from pharmaceutical companies or commercial sources may take weeks to months. The current study demonstrates that REMEDi HS can adequately provide quantitative information by using rapid estimate method.

Cartridge Lifetime and Sample Matrices

REMEDi HS is designed for continuous 24-h operation, analyzing human urine and human serum specimens. Presently, there are only urine and serum reagent kits available. Because of the rapid analysis time and the ability to screen a large number of drugs and metabolites, we sought to expand the application to other types of sample matrices. Therefore, our experience does not represent the routine operation in the emergency clinical laboratory. It is sometimes required to change cartridges more frequently when sample matrices other than human urine and human serum are used. However, there is increasing interest towards expanding the application of REMEDi HS to forensic toxicology and legal medicine. A recent report describes the development of off-line extraction procedures for forensic applications by REMEDi HS (21); this protocol may offer improved cartridge performance.

The Choice of IS in IS Method

In REMEDi HS, two ISs are used to monitor the two analytical cartridges. Therefore, the choice of IS is important for performing quantitative analysis in IS method. For example, we carried out consecutive analyses of 50 urine specimens, containing 2 μg/mL of diphenhydramine. During the analysis period, the peak heights of *N*-ethylnordiazepam (IS1), chlorpheniramine (IS2), and diphenhydramine decreased by 13.9, 6.8, and 12.3%, respectively (data not shown). Since IS1 and diphenhydramine decreased at about the same rate, the choice of IS1 would be better than IS2. This is due to the fact that neutral and weakly basic analytes are influenced more by the first analytical cartridge (reversed phase), whereas basic analytes are affected more by the second analytical cartridge (weak cation exchange). For the other drugs considered, IS2 gave superior results. If IS2 peak height is unusually higher, it is strongly suggested that the specimen probably contains the previously administered chlorpheniramine. In such a case, the specimen is analyzed once again by the use of only IS1, which means the peak of administered chlorpheniramine becomes easily discriminated from that of IS2 without any unnecessary confusion.

Conclusion

This study demonstrates that REMEDi HS is useful for a rapid estimate of drug concentrations in urine, serum, and gastric samples in addition to a rapid screening of over 500 drugs. The identification of metabolites along with the parent compounds is especially useful for the confirmation of the intake of the corresponding parent drugs. REMEDi HS is suitable for conducting TDM of ranitidine, bromisovalum, lidocaine, and diphenhydramine.

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References

(1) Patel V, McCarthy PT, Flanagan RT. Disopyramide analysis using REMEDi: comparison with EMIT and conventional high performance liquid chromatographic methods. Biomed Chromatogr, 1991;5:269–72.

(2) Ohtsuji M, Kondo T, Nishigami J, Takayasu T, Yoshita Y, Ohshima T. Analysis for drugs and poisons by REMEDi-HS® system. Japanese J Forensic Toxicol, 1995 April;13(1):39–48 (in Japanese with English abstract).

(3) Sato Y, Kurosaki K, Saito H, Kiuchi M. Analysis of methamphetamine by automated HPLC (REMEDi). Japanese J Legal Med 1995 April;48, supplement: 176 (in Japanese).
(4) Ohtsuji M, Ohshima T, Takayasu T, Nishigami J, Kondo T, Lin Z, Minamino T. Screening of antihistamine agents (diphenhydramine) with blood and urine samples by REMEDi-HS system. Proceedings of the 13th International Conference of Alcohol, Drugs and Traffic Safety; 1995;1:321–6.
(5) Kageura M, Hara K, Kashimura S, Hieda Y, Tanaka K. REMEDi HS™ for screening of drugs and poisons: comparison with GC/MS. Japanese J Forensic Toxicol, 1994 Sept;12(3):207–12.
(6) Demedts P, Wauters A, Franck F, Neels H. Evaluation of the REMEDi drug profiling system. European J Clin Chem Clin Biochem 1994 May;32(5):409–17.
(7) Chen JS, Chang KJ, Charng RC, Lai SJ, Binder SR, Essien H. The Development of a broad-spectrum toxicology screening program in Taiwan. J Toxicol Clin Toxicol 1995;33:581–9.
(8) Young NC, Tsai KS, Lai SJ. The use of a broad-spectrum drug identification system in emergency clinical toxicology screening. Presented at 25th Anniversary Meeting of Society of Forensic Toxicologists. 1995 Oct. 9–13; Baltimore, MD.
(9) Binder SR, Regalia M, McEachern B, Mazhar M. Automated liquid chromatographic analysis of drugs in urine by on-line sample cleanup and isocratic multi-column separation. J Chromatogr 1989;473:325–41.
(10) Adams AK, Essien H, Binder SR. Identification of drugs in physical fluid following on-line liquid chromatographic purification and analysis. Annales de Biologie Clinique 1991;49:291–7.
(11) Yashiki M, Miyazaki T, Chikasue F, Kojima T, Ohtani M. Detection of bromisovalerylurea in biological fluids by chemical ionization-selected ion monitoring—a case of bromisovalerylurea intoxication. Japanese J Legal Med 1987 Oct;41(5):452–6 (in Japanese with English abstract).
(12) Moffat AC. Clarke's isolation and identification of drugs. 2nd ed. London: The Pharmaceutical Press, 1986.
(13) Snook C, Otten M, Hassan M. Massive ephedrine overdosage. Veterinary and Human Toxicol, 1992;34:342.
(14) Midha KK, Cooper JK, McGilveray IJ. Simple and specific electron-capture GLC assay for plasma and urine ephedrine concentrations following single doses. J Pharmaceut Sci 1979 May;68 (5):557–60.
(15) Johnson GR. A fatal case involving hydroxyzine. J Anal Toxicol 1982 March and April;6(2):69–70.
(16) Filloux F. Toxic encephalopathy caused by topically applied diphenhydramine. J Ped 1986 June;108(6):1018–20.
(17) Gotez CM, Lopez G, Dean BS, Krenzelok EP. Accidental childhood death from diphenhydramine overdosage. Am J Emergency Med 1990 July;8(4):321–2.
(18) Koppel C, Ibe K, Tenczer J. Clinical symptomatology of diphenhydramine overdose: an evaluation of 136 cases in 1982 to 1985. J Toxicol Clin Toxicol 1987;25:53–70.
(19) Jonville AP, Barbier P, Blond MH, Boseq M, Autret E, Breteau M. Accidental lidocaine overdosage in an infant. J Toxicol Clin Toxicol 1990;28(1):101–6.
(20) Fruncillo RJ., Gibbons W, Bowman, SM. CNS toxicity after ingestion of topical lidocaine. N Engl J Med 1982 Feb;306(7): 426–7.
(21) Kalasinsky KS, Schaefer T, Binder SR. Forensic application of an automated drug-profiling system. J Anal Toxicol 1995 Oct;19: 412–8.

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