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Interference of Selected Clinical Medicines on DRI^R and TDx^R Immunoassays of Morphine and Methamphetamine in Urine

SHIOW-GUEI CHANG[1,2], CHUN-SHENG CHIEN[2], HORNG-MO LEE[1] AND CHAU-YANG CHEN[1]*

[1.] *Graduate Institute of Pharmaceutical Sciences, Taipei Medical College, 250 Wu-Hsing St., Taipei 110, Taiwan, R.O.C.*
[2.] *National Bureau of Controlled Drugs, Department of Health, Executive Yuan, Taipei 100, Taiwan, R.O.C.*

ABSTRACT

Eleven commonly used antihistamines, nonsteroidal anti-inflammatory drugs, and vitamins were evaluated *in vitro* for potential interference with DRI^R and TDx^R immunoassay reagents for opiates and amphetamines. Mechanisms for the observed interference were also explored. DRI^R reagents appear to be more susceptible to interference by the compounds studied. Regarding mechanistic aspects, tolmetin was found to be strongly absorbent at the detection wavelength (340 nm) causing a false negative response; diphenhydramine, pheniramine, and trimethobenzamide appeared to cause false positive interference through their affinity to the antibodies used in the DRI^R reagents; while chlorpromazine was found to positively interfere with the assay through both cross reaction and UV absorption. Ascorbic acid, when in high concentrations (>2%, w/v), may inhibit the enzyme (G6PDH) activity and result in a false negative response.

Key words: **immunoassay, morphine, methamphetamine, interference, *in vitro*.**

INTRODUCTION

Heroin and methamphetamine are the most commonly abused drugs in Taiwan. Mandatory urine testing for opiates and amphetamines have been or will soon be implemented for many population groups. These testing programs follow the same analytical approaches currently adopted in the U.S.[(1)], i.e., a two-step testing protocol utilizing immunoassays (IA) for preliminary screens and gas chromatography-mass spectrometry (GC-MS) methodologies to confirm those tested positive in the IA test step.

This study was conducted to address potential interference problems commonly associated with IA[(2, 3)] — one of the major concerns for a testing program adopting the two-step protocol. Specifically, antihistamines, nonsteroidal anti-inflammatory drugs, and vitamins that are commonly used in Taiwan were evaluated *in vitro* to determine whether these medicines interfere with the enzyme immunoassay (EIA), such as DRI^R, and fluorescence polarization immunoassay (FPIA), such as TDx^R, reagents commonly used for drug screening in Taiwan. Mechanisms for the observed interferences were also explored.

MATERIALS AND METHODS

Correspondence to: Chau-Yang Chen
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I. *Chemicals, Reagents, Supplies and Apparatus*

Morphine HCl was supplied by the Taiwanese National Narcotic Bureau. (+)-Methamphetamine HCl, indomethacin, tolmetin sodium, diphenhydramine HCl, brompheniramine maleate, pheniramine maleate, trimethobenzamide HCl, thiamine HCl, pyridoxine HCl, *l*-ascorbic acid, nicotinamide, and chlorpromazine HCl were purchased from Sigma Chemical Co. (St. Louis, MO). Tris-(hydroxymethyl)-aminomethane was purchased from Acros Chemical Co. (Pittsburgh, PA).

DRI^R Amphetamines (Lot Nos. 6M034, 7M067 and 8H289) and Opiates (Lot Nos. 6M088, 7C135 and 8C068) EIA reagents were from Diagnostic Reagents, Inc. (Sunnyvale, CA). FPIA TDx^R Amphetamine/Methamphetamine II (Lot No. 18263Q100) and TDx^R Opiates (Lot No. 24368Q 100) reagents were from Abbott Laboratories (Diagnostics Division, Abbott Park, IL). These reagents were tested using a Merck Vitalab Selectra 2 clinical chemistry autoanalyzer (Vital Scientific N.V., Dieren, The Netherlands), and a TDx^R system, respectively.

All GC/MS analyses were carried out using an HP-6890/HP-5972/HP 6890 Series MSD software GC-MS system (Hewlett Packard, Palo Alto, CA) equipped with a 30 m (ID, 0.25 mm; film thickness, 0.25 μm) DB-5 column (5% phenylmethylpolysiloxane). Derivatization agents, *N*-methyl-*N*-(trimethylsilyl)trifluoroacetamide and heptafluorobutyric anhydride were purchased from Aldrich Chemical Co. (Milwaukee, WI). Other instruments used included a UV-Vis spectrometer UV240 (Shimadzu, Kyoto) and an HM-7E pH meter (Toa, Tokyo).

II. *Immunoassay - Interference Detection*

Two sets of drug-containing samples (positive controls) were prepared to study potential interferences of the eleven compounds using DRI^R EIA and TDx^R FPIA reagents. Morphine or methamphetamine included in the first set were at 125% of the respective cutoffs of the drug category studied. Thus, 49.49 μg morphine HCl (37.50 μg morphine equivalent) was included in a 100 mL solution of drug-free urine. Similarly, 77.79 μg methamphetamine HCl (62.50 μg methamphetamine equivalent) was used to prepare 100 mL of methamphetamine positive control.

Samples in the second set (test samples) included the same concentrations of morphine or methamphetamine of the corresponding positive controls described above. In addition, individual interference compounds to be studied were also included individually at two different concentration levels, i.e., at half of the daily dose and the maximum daily dose (Table 1). Sample solutions at these concentrations were prepared following the method adapted by Joseph, *et al*.[4]

To detect interference, test samples and their corresponding positive controls were tested five times with both immunoassays. Means and standard deviations of these replicates were calculated. The Dunnett's test, adopting a 95% confidence level (α= 0.05, two-sided), was used as a one-way ANOVA to determine whether the observed difference between a test sample and its corresponding positive control is significant.

III. *Immunoassay — Interference Mechanism*

Based on the reaction mechanisms of the

Table 1. Two levels of the concentrations of compounds adopted for their interference

Compound studied	Low (mg/L)[a]	High (mg/L)[b]
Indomethacin	37.5	100.0
Tolmetin sodium salt	400.0	1000.0
Diphenhydramine HCl	75.0	100.0
Brompheniramine maleate	12.0	18.0
Pheniramine maleate	37.5	75.0
Thiamine HCl	10.0	150.0
Pyridoxine HCl	25.0	100.0
l-Ascorbic acid	75.0	1000.0
Nicotinamide	50.0	150.0
Chlorpromazine HCl	150.0	500.0
Trimethobenzamide HCl	375.0	500.0

[a] Concentration at half of daily dose per liter.

[b] High concentration, half of maximum daily dose per liter.

immunoassays studied, observed interference derived from the presence of the interference compounds may have originated for one or more of the following reasons (see Fig. 1): (1) pH change in the reaction medium; (2) absorption at the detection wavelength (340 nm); (3) alteration of G6PDH activity; and (4) affinity towards the antibodies. Thus, experiments were designed and carried out step by step accordingly to identify specific mechanisms for the observed interference.

Potential pH changes were studied by comparing the pH values measured from the following samples: blank urine, positive controls, and test samples containing the interfering compounds in concentrations at half of the compounds' maximum daily doses per liter. In addition, due to its low pKa, the pH of urine containing higher concentrations of ascorbic acid (2-10%, w/v) was also measured.

Potential interference due to absorption at the detection wavelength was studied by first scanning from 225 to 450 nm of the Tris buffer containing various concentrations of the interfering compounds. Absorbance at 340 nm was also measured for two sets (250 µL) of NADH-containing (300 µmol/L) Tris buffer solutions that were spiked with various concentrations of the interfering compounds studied (20 µL): one with and one without the analyte (375 µg/L morphine or 625 µg/L methamphetamine). These experiments were designed to detect, if any, absorption caused by the reaction products of the interference compounds with the analyte (morphine or methamphetamine) and/or NADH.

To study potential G6PDH activity change, 10 µL urine samples containing the interfering compounds were mixed with the following two components in a cuvette at 37°C: 125 µL Reagent A containing 40 mmol of glucose-6-phosphate and 6 mmol of NAD^+ dissolved in 1 L Tris buffer (pH

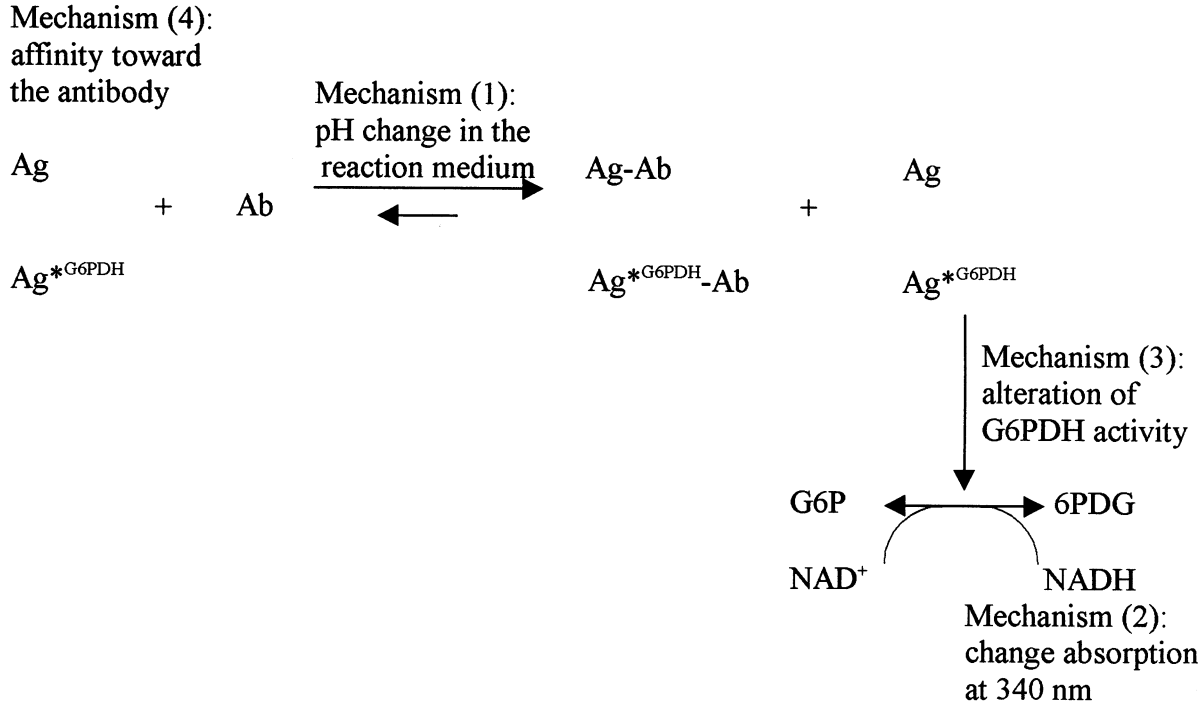

Figure 1. Scheme illustrates the interference mechanisms of DRI immunoassay reagents. Ag: the drug (analyte) to be measured; Ab: the antibody capable of binding the drug (the limiting factor in the reaction); Ag^{*G6PDH}: the drug labeled with enzyme; G6P: glucose-6-phosphate; G6PDH: glucose-6-phosphate dehydrogenase; 6PDG: 6-phospho-D-gluconate; NAD^+: nicotinamide adenine dinucleotide; NADH: nicotinamide adenine dinucleotide, reduced form.

7.8); and 125 μL Reagent B containing 40 units G6PDH dissolved in 1 L Tris buffer (pH 7.8). Changes in absorbance (ΔA) at 340 nm were measured and compared to that exhibited by the blank urine sample which was arbitrarily set to have 100% G6PDH enzyme activity. Inhibition or enhancement of G6PDH enzyme activity in various concentrations of these potentially interfering compounds was displayed by plotting the relative enzyme activity versus the concentration of the compounds studied.

Finally, cross reactivity to the antibody is considered the underlying factor when the above mechanisms (pH, absorption at the detection wavelength, and enzyme activity) are ruled out. To study the affinity of a compound toward the antibodies, a DRI^R reagent was used to assay drug-free urine containing half of the compound maximum daily dose (in 1L). For those showing significant interference, assays were also conducted at various compound concentrations to determine whether there is a direct correlation between the concentrations of the interfering compounds and the observed absorbance changes.

IV. *Extraction, Derivatization and GC-MS Analysis*

(I) *Morphine*

Nalorphine was used as the internal standard for morphine determination. Positive controls and test samples were extracted using a standard solid-phase procedure[5] and derivatized with *N*-methyl-*N*-(trimethylsilyl)-trifluoroacetamide[6]. The HP-6890/HP-5972/HP 6890 Series MSD software GC-MS system was used to collect selected ion monitoring (SIM) data using the following ions: morphine-TMS: m/z 429, 236, and 196; nalorphine-TMS: m/z 455, 440, and 260. The first ions were used for quantitative analyses using a 5-point calibration curve ranging from 50 to 1000 ng/mL.

(II) *Methamphetamine*

Methamphetamine-d_5 was used as the internal standard for the determination of methamphetamine using a standard solid phase extraction procedure[7], followed by derivatization with heptafluorobutyric anhydride. The same GC-MS system was used to collect SIM data using the following ions: methamphetamine-HFBA: m/z 254, 210, and 169; methamphetamine-d_5-HFBA: m/z 258, 213, and 92. The first ions were used for quantitative analyses using a 7-point calibration curve ranging from 50 to 3000 ng/mL.

RESULTS AND DISCUSSION

Test samples used for interference studies *in vitro* were analyzed by GC/MS procedures to ensure that the analytes (morphine and methamphetamine) were present at their expected quantities. Results obtained from test samples containing the interfering compounds at half of their maximum daily dose (per liter) are listed in Table 2. All results, with one exception, fell within ±10% of the values obtained from their respective positive controls. The reason for the elevated morphine concentration in chlorpormazine-containing sample is not known.

I. *Observed Interference*

DRI^R and TDx^R test results of positive controls and test samples are shown in Table 3. Test result differences between the test samples and their corresponding positive controls were statistically evaluated (Dunnett's test) and summarized as follows:

(I) For the two immunoassays studied, DRI^R EIA reagents appear to be more susceptible to interference with the eleven compounds studied.

(II)Trimethobenzamide appears to generate the most serious interferences to both DRI^R and TDx^R reagents, especially for the DRI^R methamphetamine assay. Chlorpromazine also causes significant interferences in both DRI^R and TDx^R assays for both drug categories studied.

(III)Other significant interferences observed were associated with the use of DRI^R reagents. These

Table 2. Results of GC/MS analysis of positive controls and test samples found to exhibit interference phenomena

Sample	Morphine[a] Mean; s.d.	Morphine[a] Recovery[b](%)	Methamphetamine[a] Mean; s.d.	Methamphetamine[a] Recovery[b](%)
Morphine (Control: 375 ng/mL)[c]	376.2; 12.8	100.0		
Morphine + tolmetin	372.0; 13.3	98.9		
Morphine + trimethobenzamide	373.7; 11.6	99.3		
Morphine + ascorbic acid	384.2; 4.9	102.1		
Morphine + diphenhydramine	392.3; 33.6	104.3		
Morphine + pheniramine	373.0; 15.7	99.1		
Morphine + chlorpromazine	542.6; 89.4	144.2		
Methamphetamine (Control: 625 ng/mL)[d]			636.6; 34.2	100.0
Methamphetamine + tolmetin			657.7; 29.7	103.3
Methamphetamine + trimethobenzamide			621.2; 52.1	97.6
Methamphetamine + ascorbic acid			624.0; 9.8	98.0
Methamphetamine + chlorpromazine			594.8; 32.7	93.4

[a] Between-run precision and accuracy were determined on three separate days, and duplicated each day.

[b] Proportion relative to positive control.

[c] Samples in this group contain 375 ng/ml morphine and the interference compound at the half of maximum daily dose per liter (see Table 1).

[d] Samples in this group contain 625 ng/ml methamphetamine and the interference compound at the half of maximum daily dose per liter (see Table 1).

Table 3. DRI and TDx test results of urine samples containing 375 ng/mL morphine (or 625 ng/mL methamphetamine) and interference compounds at two concentration levels[a]

	DRI reagents				TDx reagents			
	Morphine		Methamphetamine		Morphine		Methamphetamine	
Sample[b]	Low conc.	High conc.	Low conc.	High conc.	Low conc.	High conc.	Low conc.	High conc.
Morphine Control	394.1;6.5	389.2;7.7			394.3;5.6	392.3;4.5		
Methamphetamine Control			623.1;8.3	647.5;9.6			661.1;27.4	701.5;16.2
Indomethacin	388.7;8.3	384.9;13.1	611.0;17.9	640.3;19.9	396.9;1.7	388.6;6.2	676.9;19.5	688.6;43.5
Tolmetin sod.	377.9;3.8	360.2;6.5	578.9;21.9[c]	568.5;34.9[c]	389.6;2.0	387.6;8.5	656.1;11.5	680.8;19.4
Diphenhydramine	411.1;5.5	423.8;18.5[c]	606.8;15.5	628.7;28.1	403.6;5.2	406.1;4.6	693.0;14.5	698.5;18.1
Brompheniramine	392.9;1.9	407.0;23.9	603.7;12.8	629.2;32.9	388.2;6.6	397.3;9.1	651.0;21.9	675.7;20.8
Pheniramine	404.0;8.5	426.1;25.3[c]	606.3;11.3	630.6;28.0	401.3;4.7	405.5;4.1	686.5;9.2	672.5;31.7
Thiamine	389.7;22.6	392.7;9.9	604.6;14.4	637.5;30.1	387.2;2.0	396.8;7.1	676.0;14.2	674.0;40.6
Pyridoxine	386.3;15.8	386.3;17.4	605.4;21.7	626.0;34.7	388.7;8.5	391.3;9.7	676.0;6.4	634.2;35.9
Ascorbic acid	379.5;11.3	378.7;17.7	604.6;17.1	618.6;23.4	385.1;11.1	393.6;7.2	672.2;6.3	637.4;19.3
Nicotinamide	387.0;19.3	385.5;15.6	612.9;15.8	637.4;30.5	395.3;3.1	398.4;9.7	682.4;30.1	697.2;35.2
Chlorpromazine	603.7;36.4[c]	805.9;22.5[c]	650.0;13.5	715.4;33.2[c]	458.7;5.4[c]	519.0;14.0[c]	785.3;26.0[c]	871.1;17.1[c]
Trimethobenzamide	451.3;20.6[c]	475.2;22.8[c]	4889.4;99.9[c]	5513.6;138.9[c]	425.3;3.3[c]	433.6;4.8[c]	2460.4;89.3[c]	2666.8; 76.8[c]

[a] Low concentration is equivalent to half of the respective drug's daily dose per liter; high concentration is equivalent to half of maximum daily dose per liter (see Table 1 of numerical values).

[b] Numbers in the body of the table are in ng/mL, means and standard deviation of 5 replicates.

[c] Figures were found to be significantly different (Dunnett's test, $\alpha = 0.05$, two-sided) from their respective controls.

include the assay of methamphetamine in the presence of tolmetin (at both concentrations) and the assay of morphine in the presence of diphenhydramine and pheniramine at the higher concentration level.

II. *Interference Mechanism*

Since DRI® reagents were found to be more susceptible to interference by the compounds studied, DRI® reagents were used for mechanistic studies with a focus on the effects of the interference compounds on medium pH, spectrometric measurement, enzyme activity, and affinity toward antibodies.

(I) *Effects of Interfering Compounds on the pH of the Reaction Media*

With the exception of ascorbic acid, the presence of these tested compounds at a higher concentration level studied did not alter the pH of the morphine-containing test samples; all within a pH range of 6.60 to 6.70. Presence of ascorbic acid at the higher concentration level changed the medium pH to 6.00, which is still within the acceptable range specified by DRI® reagent. Schwarzhoff and Cody[8] reported that 10% ascorbic acid caused a drastic change in urine pH to result in false EIA test results. To verify their study, a series of test samples containing 2-10% of ascorbic acid were prepared. These test samples were then mixed with the Tris buffer (pH 7.8) in 10:250 ratio. The pH of these mixtures and DRI® test results are shown in Table 4. The pH of the reaction medium can still be adjusted by Tris buffer to the range of 5.0-8.0, acceptable to EIA reagents [9], up to 5% w/v of ascorbic acid. However, significantly reduced DRI® test results were observed even when ascorbic acid was at the 2% level. Thus, the observed interference must have been caused by factors other than medium pH and will be explained in a later section.

(II) *Interference through Absorption at the Detection Wavelength*

UV absorption spectra shown in Fig. 2 indicate strong absorption of tolmetin at 340 nm, the wavelength adopted for the detection of NADH by the EIA methodology. Significant deviations

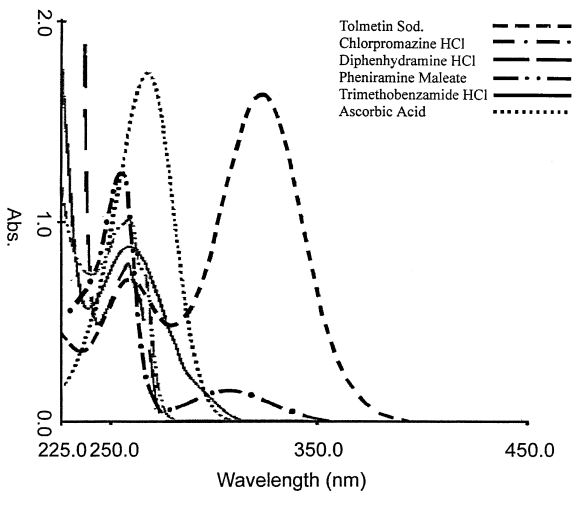

Figure 2. UV spectra (225-450 nm) of interference compounds in Tris buffer solution. Concentrations: diphenhydramine HCl, 750 mg/L; pheniramine maleate, 60 mg/L; ascorbic acid, 60 mg/L; trimethobenzamide HCl, 55 mg/L; chlorpromazine HCl, 40 mg/L; tolmetin sod., 27 mg/L.

Table 4. The pH and DRI immunoassay results of urines adulterated with various concentrations of ascorbic acid

Sample	pH		Morphine	Methamphetamine
	Urine	Urine/Tris buffer (1:25)	Mean; %	Mean; %
Blank urine	6.50	7.8	— —	
Positive control urine[a]	6.50	7.8	374;100.0	616;100.0
With 2% ascorbic acid[a]	3.90	7.6	171; 45.7	337; 54.6
With 5% ascorbic acid[a]	3.45	7.0	-132;-77.4	-204; -60.5
With 10% ascorbic acid[a]	3.10	4.5	—	—

[a] Containing 375 ng/mL morphine and 625 ng/mL methamphetamine.

from the Lambert-Beer law were observed when the concentration of tolmetin was higher than 200 mg/L. Therefore, in the presence of tolmetin, absorbance changes are not in proportion to the concentration of the abused drug and may result in a false negative response. This is the most likely cause for the negative tolmetin interference observed in Table 3. This explanation is consistent with research reporting[4] that tolmetin caused false negative results for EMIT assays of opiates and cannabinoids.

An absorption spectrum of chlorpromazine HCl (Fig. 2) also indicated absorption at 340 nm, which becomes very significant at higher chlorpromazine concentrations. This finding is consistent with previous studies[10,11].

A closer examination of data in Fig. 3 reveals a 15% increase in UV absorbance in NADH Tris buffer spiked with 500 mg/L of chlorpromazine (half of maximum daily dose per L). However, a two-fold increase in opiate concentration was observed at this chlorpromazine concentration level. Thus, mechanisms other than absorption at the detection wavelength might have been responsible for the interference of chlorpromazine observed in the DRI Opiate Immunoassay.

(III) *Alteration of G6PDH Activity*

Experiments designed for this study exclude the antibody from the reaction mixture, thus, ruling out the effects derived from antibody affinity. As stated earlier, our observation could not attribute the interference of ascorbic acid to change in pH of the reaction medium as reported by earlier studies[2, 8]. Serious interference was observed long before the pH of the reaction media was out of the acceptable range specified for the proper function of the reagents. Interference is more likely caused by the inhibition of NADH formation with ascorbic acid serving as a competing reducing agent. This reasoning is supported by the fact that the standard reduction potential of ascorbic acid is larger than that of NADH [12], thus inhibiting the conversion of NAD^+ to NADH.

The apparent inhibition effect of tolmetin toward G6PDH activity as shown in Fig. 4 may not be real. Since tolmetin exhibits high absorption at 340 nm (Fig. 2), the extremely high absorption of the test sample in the presence of high tolmetin concentration at t2 might have

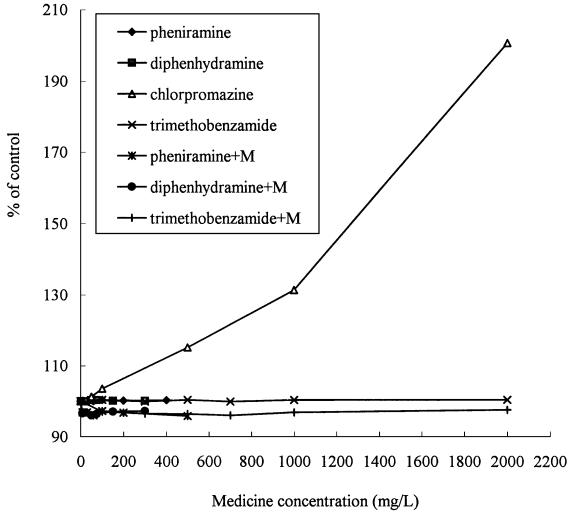

Figure 3. Absorbance of NADH at 340 nm with different concentrations of interference compounds. Concentrations: trimethobenzamide, 100-2000 mg/L; diphenhydramine, 10-300 mg/L; pheniramine, 10-400 mg/L; chlorpromazine, 10-2000 mg/L; morphine (M), 375 μg/L.

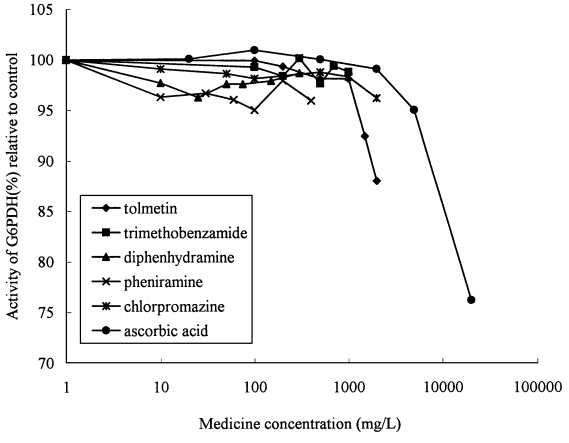

Figure 4. Activity of G6PDH inhibited by different concentrations of interference compounds. Solution: Reagent A: Reagent B = 10μL: 125μL : 125μL. Concentrations: tolmetin, 100-2000 mg/L; trimethobenzamide, 100-1000 mg/L; diphenhydramine, 10-300 mg/L; pheniramine, 10-400 mg/L; chlorpromazine, 10-2000 mg/L; ascorbic acid, 20-20000 mg/L.

exceeded the applicable range (Abs. 3.0) of the analyzer. Thus, the interference mechanism for tolmetin is most likely due to its absorption at the detection wavelength. This explanation is consistent with reports on the reduced responses of Syva EMIT reagents in the presence of salicyluric acid, an aspirin metabolite [9, 13, 14]. The observed interference was first attributed to inhibition of enzyme activity, but later to spectrometric absorption at 340 nm [9, 13, 14].

(IV) *Affinity between Interference Compounds and the Antibody*

Diphenhydramine, pheniramine, and trimethobenzamide do not absorb at 340 nm (Fig. 2), nor do they interact with morphine to interfere the UV absorption (Fig. 3). However, presence of these compounds do affect the assay results.

Data in Table 5 indicate significant false detection of morphine and methamphetamine by DRI® reagents in the presence of chlorpromazine and a drastic false positive response of methamphetamine in the presence of trimethobenzamide. Other compounds that were found to cause minor false detection of morphine are diphenhydramine and pheniramine. Interference of these compounds were further studied at various concentration levels (Fig. 5), which further confirmed the interference nature of these compounds. Since none of these compounds showed absorbance at 340 nm (except chlorpromazine), caused pH change of the media, or altered G6PDH activity, the observed interfering phenomenon caused by the presence of these compounds is attributed to their affinity toward the antibodies of the test reagents.

In conclusion, among the eleven tested adulterants, tolmetin interfered with the DRI® methamphetamine immunoassay through UV absorption at the detection wavelength resulting in false negative responses. Diphenhydramine HCl, pheniramine maleate, and trimethobenzamide HCl interfered with DRI® immunoassay through cross reaction with the antibodies in the assay reagents. Chlorpromazine HCl interfered with the assay through both cross reaction and UV absorption. Reducing agents such as ascorbic acid, when at high concentrations, may inhibit the activity of G6PDH, causing the inhibition of the conversion of NAD^+ to NADH. Interference resulting from

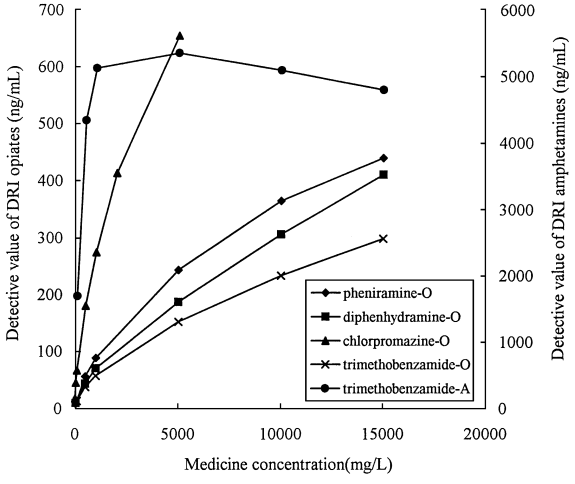

Figure 5. Affinity of interference compounds toward antibody in DRI opiates (O) or amphetamines (A) reagents. Concentrations: pheniramine-O, 50-15000 mg/L; diphenhydramine-O, 50-15000 mg/L; trimethobenzamide-O, 100-15000 mg/L; tirmethobenzamide-A, 100-15000 mg/L; chlorpromazine-O, 10-5000 mg/L.

Table 5. DRI test results of urine samples containing only the interference compound (at their half of maximum daily dose per liter)[a]

Sample	Opiates	Amphetamines
Morphine	394.3; 19.0	—
Methamphetamine	—	612.6; 26.5
Blank urine	0.7; 1.0	2.1; 0.6
Indomethacin	-7.1; 6.0	-9.2; 5.6
Tolmetin sod.	-9.1; 4.8	-35.6; 6.3
Diphenhydramine	16.7; 2.8	-4.4; 4.1
Brompheniramine	1.7; 5.9	-4.6; 6.1
Pheniramine	15.3; 2.6	-5.5; 6.0
Thiamine	-1.2; 5.1	-5.1; 3.6
Pyridoxine	-7.5; 6.5	-17.1; 4.3
Ascorbic acid	-4.3; 3.5	-23.3; 7.9
Nicotinamide	-6.8; 8.7	-11.9; 7.7
Chlorpromazine	186.1; 11.9	102.8; 20.1
Trimethobenzamide	35.0; 1.0	4262.6; 36.3

[a] Mean and standard deviation of triplicates.

pH change of the reaction medium is not a significant factor for the compounds studied.

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REFERENCES

1. Hawks, R. L. 1986. Analytical methodology. In "Urine Testing for Drugs of Abuse". Research Monograph Series 73. pp. 30-42. Hawks, R. L. and Chiang, C. N. ed. National Institute on Drug Abuse (NIDA), Department of Health and Human Services, Rockville, Md., U.S.A.
2. Mikkelsen, S. L. and Ash, K. O. 1988. Adulterants causing false negative in illicit drug testing. Clin. Chem. 34: 2333-2336.
3. Cody, J. T. and Schwarzhoff, R. H. 1989. Impact of adulterants on RIA analysis of urine for drugs of abuse. J. Anal. Toxicol. 13: 277-284.
4. Joseph, R., Dickerson, S., Willis, R., Frankenfield, D., Cone, E.J. and Smith, D.R. 1995. Interference by nonsteroidal anti-inflammatory drugs in EMIT® and TDx® assays for drugs of abuse. J. Anal. Toxicol. 19: 13-17.
5. Hold, K. M., Wilkins, D. G., Rollins, D. E., Joseph, Jr. R. E. and Cone, E. J. 1998. Simultaneous quantitation of cocaine, opiates, and their metabolites in human hair by positive ion chemical ionization gas chromatography-mass spectrometry J. Chromatogr. Sci. 36: 125-130.
6. Polettini, A., Groppi, A. and Montagna, M. 1993. Rapid and highly selective GC/MS/MS detection of heroin and its metabolites in hair. Forensic Sci. Int. 63: 217-225.
7. ANSYS Diagnostics, Inc. 1996. SPEC extraction of amphetamine and methamphetamine. In "Automated SPEC® Solid Phase Extraction Protocols for Drugs of Abuse". pp. 69-76. ANSYS, Inc. Irvine, CA., U.S.A.
8. Schwarzhoff, R. and Cody, J.T. 1993. The effects of adulterating agents on FPIA analysis of urine for drugs of abuse. J. Anal. Toxicol. 17: 14-17.
9. Linder, M. W. and Valdes, R. Jr. 1994. Mechanism and elimination of aspirin-induced interference in Emit® II d.a.u. Assays. Clin. Chem. 40: 1512-1516.
10. Poklis, A. 1992. Unavailability of drug metabolite reference material to evaluate false-positive results for monoclonal EMIT® d.a.u.TM assay of amphetamine. Clin. Chem. 38: 2560.
11. Olsen, K. M., Gulliksen, M. and Christophersen, A. S. 1992. Metabolism of chlorpromazine and brompheniramine may cause false-positive urine amphetamine results with monoclonal EMIT® d.a.u. TM immunoassay. Clin. Chem. 38: 611-612.
12. Harris, D. C. 1995. Quantitative chemical analysis. 4th ed. pp. 366-369. W. H. Freeman and Company, New York, U.S.A.
13. Wagener, R. E., Linder, M. W. and Valdes, R. Jr. 1994. Decreased signal in EMIT assays of drugs of abuse in urine after ingestion of aspirin: Potential for false-negative results. Clin. Chem. 40: 608-612.
14. Linder, M. W. and Valdes, R. Jr. 1994. Mechanism of aspirin induced interference in EMIT d.a.u. assay: A solution to this problem. J. Clin. Immunoassay 17: 59.

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探討醫療藥物影響DRI^{R} 及TD$_{X}^{R}$ 免疫試劑檢測尿中嗎啡及甲基安非他命

張秀圭[1,2]　簡俊生[2]　李宏謨[1]　陳朝洋[1]

[1.] 臺北醫學院藥研所　台北市信義區吳興街250號
[2.] 行政院衛生署管制藥品管理局

摘　要

本實驗篩選國內醫療常用藥品分別類屬於抗組織胺、非類固醇抗發炎藥、維他命製劑等共十一種藥物進行體外試驗，評估該等藥品對DRI^{R} 及TD$_{X}^{R}$ 免疫試劑檢測尿液中嗎啡與甲基安非他命干擾之可能性，同時探究DRI^{R} 試劑受干擾現象之原因。實驗結果顯示tolmetin於紫外線波長340nm有強吸收導致造成偽陰性結果； diphenhydramine、pheniramine、trimethobenzamide三者可能與DRI^{R} 試劑之抗體具親和力引起交叉反應；至於chlorpromazine造成偽陽性反應之可能原因包括：於紫外線波長340nm有吸收及與DRI^{R} 試劑之抗體具親和力兩者；ascorbic acid 於溶液中濃度大於2%時有可能抑制G6PDH酵素引起DRI^{R} 試劑偽陰性反應。

關鍵詞：免疫分析，嗎啡，甲基安非他命，干擾，體外試驗。