VARIATIONS OF METHYLEPHEDRINE CONCENTRATION IN URINE AFTER TAKING OTC COLD MEDICINE

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ABSTRACT

Methylephedrine (MEP), a drug banned by the International Olympic Committee (IOC), is frequently found in over-the-counter (OTC) cold medicines in Taiwan to relieve nasal congestion or a runny nose. The present study monitored the variations of MEP concentration in urine, using a simple and rapid HPLC assay method, after subjects took OTC cold medicine marketed in Taiwan. Information garnered from this study can help athletes to avert unexpected MEP-positive detection in the urine due to the administration of these cold medicines. The HPLC system consisted of an Cosmosil 5C_{18}-MS-Ⅱ column (4.6×250 mm i.d.) using a mobile phase of 0.05M phosphate buffer (pH=3.5): acetonitrile(85:15, v/v) with UV detection at 206 nm. From the intra- and interday tests, the coefficients of variations (CVs) were between 0.97% and 3.66% for the former and 1.95% and 5.07% for the latter. Accuracy was found to be between 98.52% and 104.77%. The MEP in the four OTC cold medicines used in this study was determined to be 99.08~101.27% of the declared content. After taking a single dose of Feng-re-you cold liquid (4.95mg), Zentoru capsule (14mg) or Gwoam cold liquid (7.425mg), the maximum concentration of MEP in the urine was 3-7μg/ml within 20 hours, while that of Si-si capsules (25mg) revealed MEP-positive detection, whose maximum concentration was much higher than the cut-off value of 10μg/ml set by the IOC. Thirty hours after the administration of the Si-si capsule, the concentration of MEP in the urine was below the cut-off value. The results suggest that athletes must avoid administration of MEP-containing OTC cold medicine before competition. If it is necessary to take OTC cold medicine to relieve the symptoms of a cold, athletes should select cold medicines whose MEP content is below 14 mg. Athletes who wish to take OTC cold medicine with an MEP content of 14 mg or higher, should take the medicine at least 30 hours before competing to avoid the possibility of an MEP-positive drug test.

Key words: methylephedrine, OTC, HPLC, cold medicine

INTRODUCTION

Numerous local and international sport competitions are held routinely around the world. In order to ensure that athletic performance is not tainted by the use of performance-enhancing substances, the International Olympic Committee (IOC) has made every effort to identify and ban athletes who take performance-enhancing drugs. Examples of illegal substances include stimulants, narcotic analgesics and anabolic steroids. In some cases, athletes unintentionally consume a banned substance when they take medication to treat a genuine medical condition. Athletes in this situation might test positive for a banned substance, which might lead to exclusion from competition or the for feiture of a medal. For example, the Romanian gymnast Andreea Raducan lost her gold medal at the Sydney Olympic Games in 2000 because of a positive drug test. Ms. Raducan had taken cold medicine containing ephedrine (EP) congeners, which have been banned in sports competition by the IOC since 1967 due to their sympathomimetic activity. Methyledphedrine (MEP), one type of ephedrine congener, is frequently included in over-the-counter (OTC) cold medicines in Taiwan to relieve nasal congestion or a runny nose. In order to help athletes avoid unexpected MEP-positive detection in the urine due to administration of cold medicines, the present study monitored the variations in MEP concentration in the urine after ingestion of OTC cold medicines marketed in Taiwan.

MATERIALS AND METHODS

Materials

The MEP was obtained from the National Bureau of Controlled Drugs, Department of Health, Taiwan, R.O.C, and LC grade CH_3CN was obtained from BDH. Feng-re-you cold liquid, Zentoru capsules, Gwoan cold liquid and Si-si capsules were purchased from local drugstores. The Cosmosil $5C_{18}$-MS-II reverse-phase HPLC column (250 × 4.6 mm i.d.) is a product of Nakalai Tesque (Kyoto, Japan). All other chemicals were of analytical reagent grade.

Volunteers and study design

Four healthy males (26 ± 2 years old; 69 ± 8 kg) passed the brief physical examination, and signed an informed consent agreement prior to participating in the study. The subjects were asked to avoid taking any medication, coffee, tea or alcohol two weeks before and during the study. A randomized, crossover study design with four phases was used. The subjects were given one of four OTC cold medicines in a labeled single dose for adults during each phase. A one-week washout phase occurred between ingestion of each of the four medicines. The labeled single dose for adults of Feng-re-you cold liquid, Zentoru capsule, Gwoan cold liquid and Si-si capsule was 4.95mg (10ml), 14mg (a capsule), 7.425mg (30ml) and 25mg (a capsule), respectively.

On test days, participants had a standard breakfast without caffeinated beverages, and took the medicine at 8:30 AM. After ingestion of the medication, all urine produced within 72 hours was collected and stored at -20°C. In order to imitate the a doping control situation in sports, all urinary samples from this study were collected when the subjects want to urinate naturally, and the subjects kept to their normal daily activities, including food, drink and exercise.

HPLC assay conditions and sample treatment

A Shimadzu LC-10AT HPLC pump system equipped with an Sil-10A autoinjector, CBM-10A communications bus module, DGU-4A degasser, CTO-10A column oven, and SPD-M10AVP diode array detector was used to analyze MEP in the urine and OTC cold medicines on a Cosmosil $5C_{18}$-MS-II column (i.d. 4.6 × 250 mm) at 37°C and 206 nm. A mixture of 0.05M phosphate buffer (pH=3.5)/CH_3CN (85/15, v/v) was used as the mobile phase at a flow rate of 1 ml/min and an injection volume of 50μl. Methamphetamine (MEAP) was used as an internal standard.

A standard curve for MEP in marketed cold medicines was carried out in the range of 0.5 μg/ml to 100 μg/ml in 50% aqueous methanol containing 12.35 μg/ml of MEAP as an internal standard. A single dose of each marketed cold medicine for adults was adjusted to 100ml with 50% aqueous methanol. After sonication in an ultrasonic bath (branson 5200) and filtration (0.45 μm, millipore), 10ml filtrate was diluted to 100ml using 50% aqueous methanol and then subjected to HPLC analysis.

A standard curve for MEP in urine were conducted by spiking a stock solution of MEP (1000μg/ml) into blank urine to become a concentration ranging from 0.5 to 100μg/ml with 9 μg/ml of MEAP as an internal standard. The extration procedure of a urine sample was performed according to the method reported by van der Merwe, Brown, and Hendrikz (1994). Briefly, a mixture containing 1 ml of urine from a subject and 10μl of MEAP (1000μg/ml) were prepared and alkalified by adding 100μl of 20% NaOH solution. The mixture was extracted with 4ml diethyl ether by a vortex for 30 seconds and then centrifuged at 1500×g for 5 minutes. The organic layer was collected and further acidified by adding 100μl of 1% acetic acid solution. The acidified solution was vortex-mixed again for 30 seconds, and was centrifuged at 1500×g for 5 minutes. The remaining ether in aqueous layer was evaporated under a soft stream of high purity nitrogen. Finally, the obtained aqueous layer was diluted with 100μl of mobile phase, and then was subjected to HPLC analysis.

RESULTS AND DISCUSSION

Validation of the HPLC method

In order to estimate MEP in marketed cold medicine and in the urine of the subjects after ingestion of the medications, an HPLC method was developed. The HPLC chromatograms of the MEP standard and that in the subjects' urine after taking cold medicines are shown in Figs.1A-B. As shown in Fig.1A, the retention time of MEP was about 6.3 min with detection limits of 0.01μg/ml (S/N > 3). To avoid potential interference, MEAP with a retention time of 8.8 min was chosen as an internal standard (Fig.1A). It has been reported that about 8 % of MEP metabolized to EP within 24 hrs, while about 32 % of free form MEP can be found in the urine within a 24-hour period (Wilkinson & Beckett, 1968). The peak of EP, the minor metabolite of MEP, was also found in HPLC chromatograms of urine after subjects took OTC cold medicine containing MEP; the concentration of EP in the urine was always much less than that of MEP. Therefore, we monitored mainly the variations of MEP in the urine after our subjects ingested the OTC cold medicines. The calibration curve was constructed by plotting the MEP–MEAP response area ratio on the HPLC chromatogram vs. MEP concentration, which ranged from 0.5~100μg/ml. The square of the correlation coefficients of the linear regression analysis was higher than 0.9997. As shown in Table 1, the coefficients of variability (CV) for intra- and inter-day for five replicate determinations for five consecutive days at 1, 5 and 20 μg/ml were between 0.97% and 3.66% for the intra-day and 1.95% and 5.07% for the inter-day. The accuracy at concentration at 1, 5 and 20 μg/ml ranged from 98.52 to 104.77 %. According to these results, the developed HPLC method was judged to be reliable and applicable for quantifying MEP in marketed cold medicine and urine after taking these medicines.

Table 1 Intra- and Inter-day Analytical Precision, and Accuracy for MEP.

Spiked conc. (μg/mL)	Intraday[a]			Interday[b]			Accuracy (%)
	Mean	Std dev	%CV	Mean	Std dev	%CV	
1	1.01	0.04	3.66	1.05	0.05	5.07	104.71
5	4.94	0.05	1.02	4.93	0.10	1.95	98.52
20	20.84	0.20	0.97	20.95	0.45	2.14	104.77

n=5 b.n=25

Quantitation of MEP in marketed cold medicine and variations of that in urine after taking these medicines

After validation, the developed HPLC method was applied to quantify the MEP content of cold medicine on the market and variations of that in urine after taking these medicines. As shown in Table 2, MEP in four marketed cold medicines including two capsules and two cold liquids were 99.08~101.27% of the declared content. The accuracy of MEP in these OTC cold medicines was close to 100 % of the declared content.

Table 2 The Content of MEP in Four OTC Cold Medicines. (n=9)

OTC cold medicines	Content declared (mg)	Found value (mg)	Accuracy (%)
Si-si capsule	25.00	24.77	99.08
Zentoru capsule	14.00	13.96	99.71
Gwoam cold liquid	7.425	7.52	101.27
Feng-re-you cold liquid	4.95	4.92	99.39

The variations of MEP in urine after administration of Feng-re-you cold liquid (4.95mg), Zentoru capsule (14mg), Gwoam cold liquid (7.425mg), and Si-si capsule (25mg) were further monitored. As shown in Figs.2A-D, the Cmax of MEP in the urine within 20 hours after taking these cold medicines, was between 3~18μg/ml. Reasonable correlation was found between Cmax in the urine and the dose at administration. In addition, we also found an abrupt increase in MEP in the urine 24 hours after ingestion of Zentoru capsules and Feng-re-you liquid. The results may be attributed to a urine-concentrated effect during sleep. The IOC regulated the cut-off concentration at 5μg/ml (increased to 10μg/ml in 2001) for MEP and EP. According to this criterion, only the much Si-si capsule (25mg) revealed MEP-positive detection, whose Cmax was much higher than the cut-off value of 10μg/ml. Thirty hours later, the concentration of MEP was below the cut-off value.

From a practical point of view, athletes must avoid administration of MEP-containing OTC cold medicine before competition. If it is necessary to take OTC cold medicine to relieve the symptoms of a cold, the athlete should select the cold medicines with an MEP content below 14 mg. If an athlete wants to take an OTC cold medicine with an MEP content of 14 mg or higher, the athlete should take the drug at least 30 hours before competing..

Based on our results, we speculate that ingestion of OTC cold medicines might have caused an MEP concentration over the cut-off concentration (5μg/ml, before 2001), even if the athlete only took 7mg of MEP hydrochloride. To correspond with reasonable medical treatment, the IOC lifted the cut-off concentration to 10μg/ml for MEP in 2001, but this study still showed the concentration would be too high (greater than 10μg/ml) after ingestion of a single dose of OTC cold medicine (containing 25mg MEP hydrochloride). Furthermore, people seldom take medicines only once a day when they are sick. In general, it is recommended that the medicine be taken three times a day, and this raises the probability of a positive drug test. In addition to the commercially prepared cold medicines, the folk prescription using with prescription form was usually made in 25mg tablet, the MEP dosage is the same with Si-si capsule. Besides, there are many Chinese herbal preparations which contain Mahuang (Ephedra). Mahuang is a traditional Chinese medicinal herb and its major medicinal components are ephedrine and pseudoephedrine (Okamura, Miki, Harada, Yamashita, Masaoka, Nakamoto, Tsuguma, Yoshitomi, & Yagi, 1999; Sagara, Oshima, & Misaki, 1983). And about 2% ephedrines in Mahuang were found (Sagara et al., 1983; Zhang, Tian, & Lou,

1988). Generally, one preparation of Chinese herbal medicine contains about 2~3g of Mahuang. This means that one would ingest about 40~60mg of ephedrines from a single dose of Chinese herbal medicine. Athletes who use Chinese herbal medicines might face a positive drug test, if they consume these medicines prior to competition. A Dutch professional cyclist consumed a liquid herbal food supplement with Mahuang as one of its 15 declared ingredients and tested positive for norpseudoephedrine (Ros, Pelders, & Desmet, 1999). This evidence indicates that there are significant risks associated with taking OTC cold medicines.

In order to imitate actual drug testing in sports, all urine samples in this study were collected when the subjects wanted to urinate naturally. In this study the subject's fluid ingestion was not controlled, but all urinary samples pH (Fig.3) and specific gravity (Fig.4) from this study conformed to doping control procedures (pH: 5~7.5; specific gravity: $\geqq$1.010). Since the sampling schedule didn't consider time factors, the exact time of the peak urinary MEP concentration is unclear. In this study, it appeared the highest concentration occurred within 24 hours after administration. However, many factors affect the metablism of ephedrines, such as the urinary pH value (Wilkinson & Beckett, 1968). Furthermore, other evidence has indicated that the pharmacokinetic response in highly in trained athletes may be different from the general population (Boel, Anderson, Rasmussen, Hansen, & Dossing, 1984; Eddington, Adekoya, & Kharidia, 1998). The variations in MEP pharmacokinetic response between highly trained athletes and the average person should be explored in future studies.

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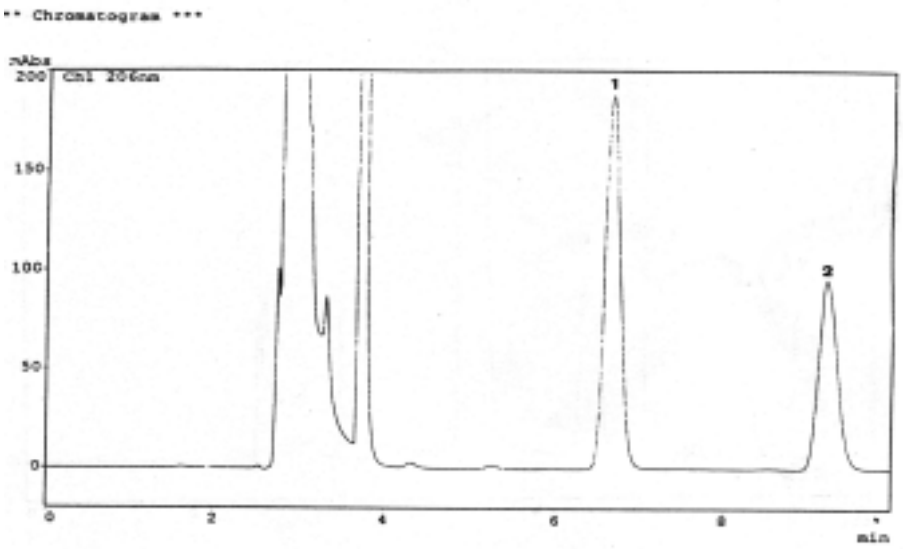
Zhang, J., Tian, Z., & Lou, Z. (1988). Simultaneous determination of six alkaloids in Ephedrae Herba by high performance liquid chromatography. *Planta. Medica., 54*, 69-70.

A



B

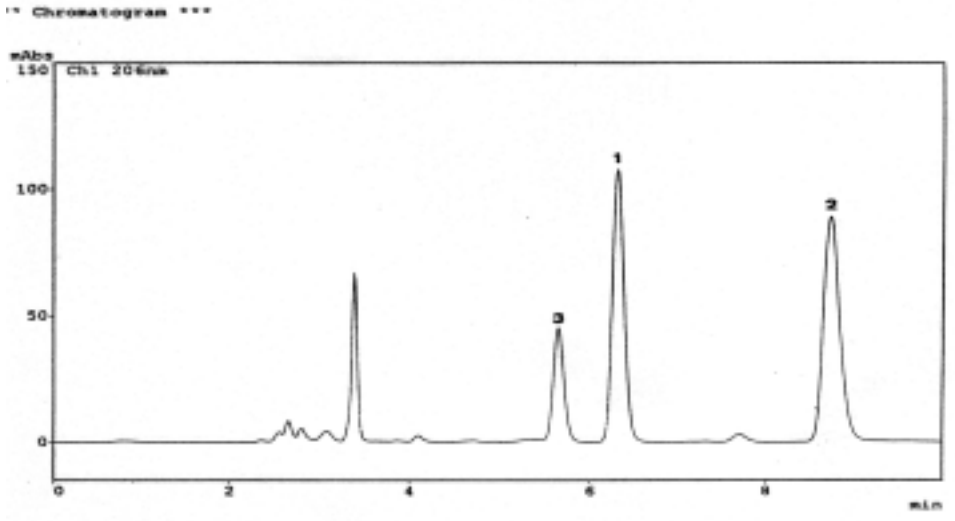
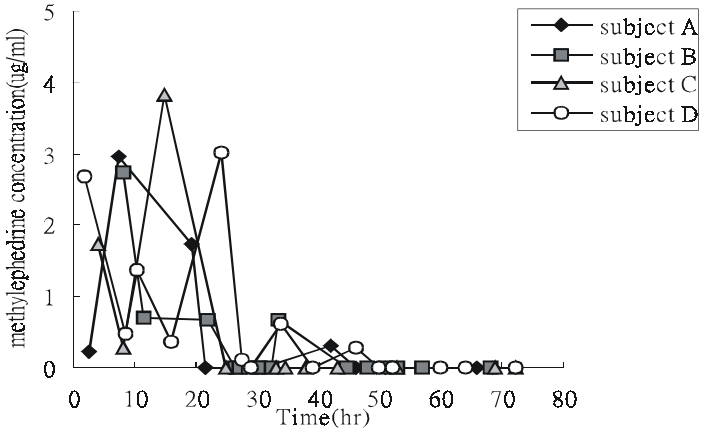
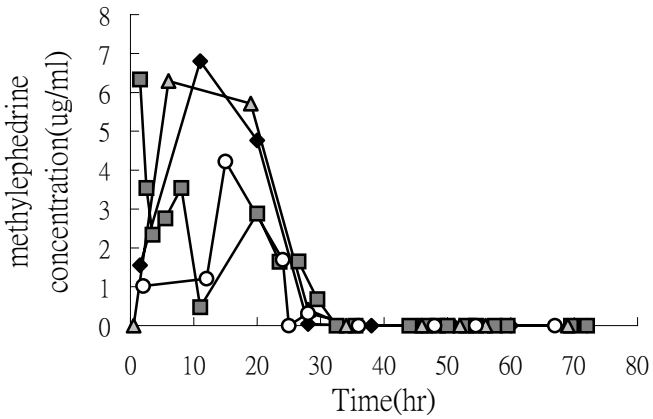


Fig.1 HPLC chromatograms of MEP standard(A) and that in urine after taking cold medicines (B).
1=methylephedrine 2=internal standard 3=ephedrine

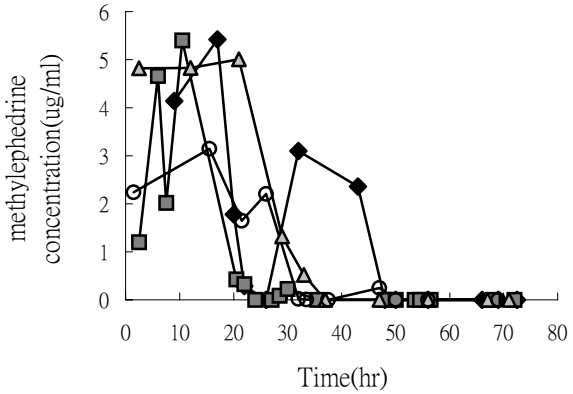
A


subject A
subject B
subject C
subject D
methylephedrine concentration(ug/ml)
Time(hr)

B


methylephedrine concentration(ug/ml)
Time(hr)

C


methylephedrine concentration(ug/ml)
Time(hr)

D

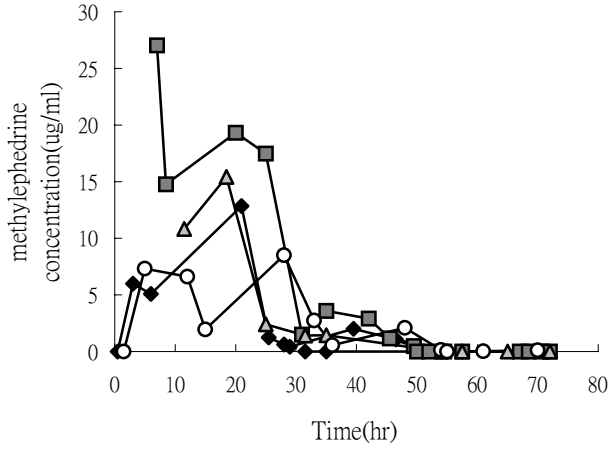
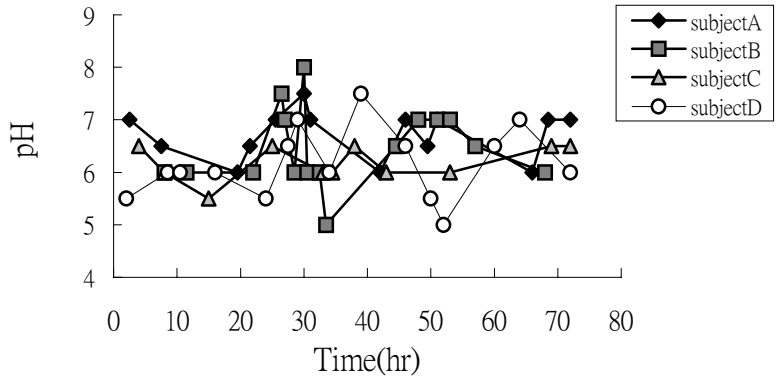


Fig. 2. Variations of MEP in urine after taking Feng-re-you cold liquid (A), Gwoam cold liquid (B), Zentoru capsule (C), & Si-Si capsule (D)

A



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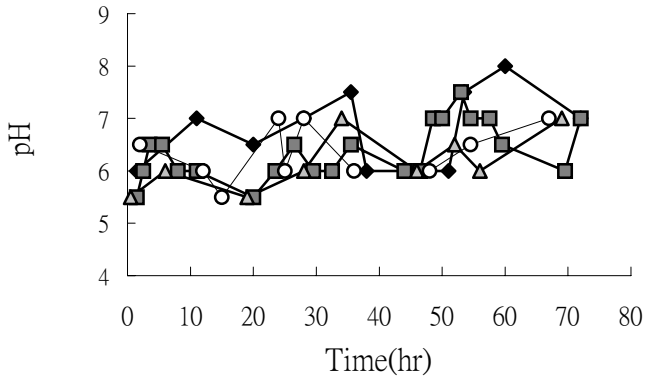

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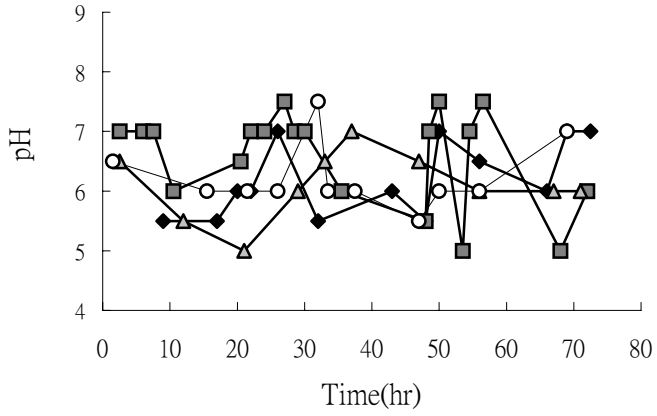

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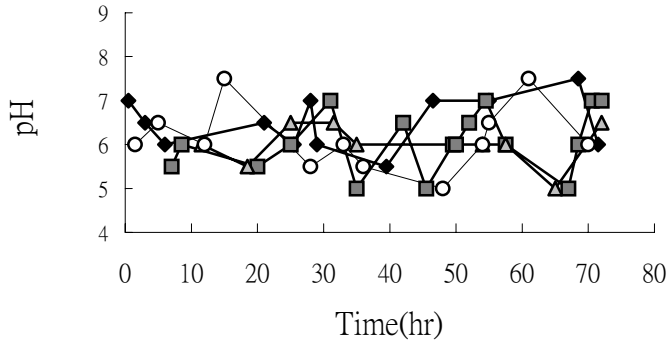
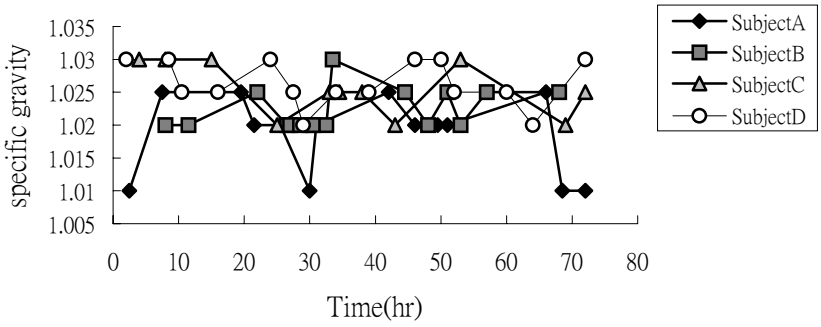


Fig. 3. Variations in urine pH after taking Feng-re-you cold liquid (A), Gwoam cold liquid (B), Zentoru capsule (C), & Si-Si capsule (D)

A



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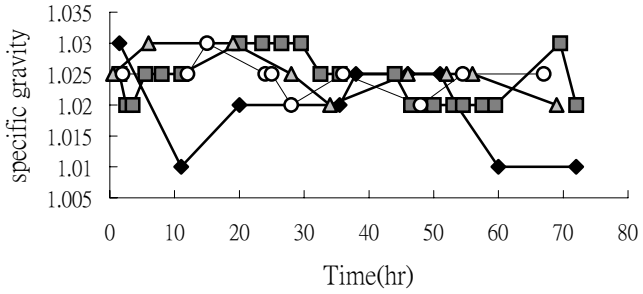

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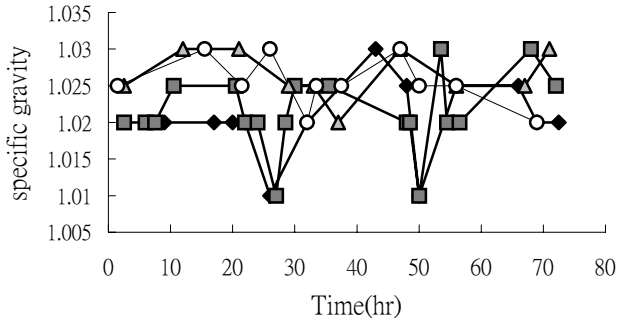

D

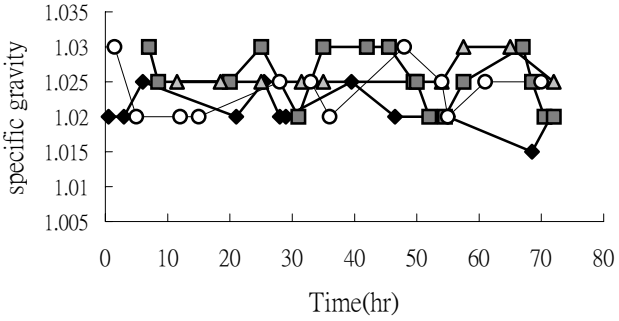

Fig. 4. Variations in urine specific gravity after taking Feng-re-you cold liquid (A), Gwoam cold liquid (B), Zentoru capsule (C), & Si-Si capsule (D)

口服甲基麻黃鹼後尿液中之含量變化

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摘 要

甲基麻黃鹼為國際奧林匹克委員會(IOC)所禁用之興奮劑之一種。在台灣有許多感冒成藥添加此成份來舒解鼻塞和流鼻涕等症狀。本研究建立一簡單而快速的高效液相層析法，來檢測市售複方感冒藥與服用這些藥物後尿液中甲基麻黃鹼之含量，以避免運動員服用後，造成藥檢陽性反應。此方法之分離管柱為 Cosmosil $5C_{18}$-MS-Ⅱ(250×4.6mm I.D.) ，移動相溶液為 0.05M 的 phosphate buffer(pH=3.5) 及 CH_3CN (85:15, v/v)，流速 1.0 ml/ min, 以紫外光吸收波長 206nm 偵測，分離管溫度設定為 37℃時，可在 10 分鐘內完成分析。在再現性測試上，同日變異係數介於 0.97%與 3.66%之間，異日變異係數介於 1.95%與 5.07%之間，準確度介於 98.52%至 104.77 之間。斯斯咳嗽膠囊、全多祿感冒膠囊、國安感冒液、風熱友感冒液等四種市售複方感冒藥，經分析後發現甲基麻黃鹼含量約為商品標示含量之 99.08~101.27%。收集健康男性受試者口服上述市售複方感冒藥單次劑量後之尿液，結果發現服用風熱友感冒液(4.95mg)、全多祿感冒膠囊(14mg)、國安感冒液(7.425mg)後，受試者尿液中之甲基麻黃鹼在 20 小時內達到其最高濃度 3~7μg/ml；而服用單次劑量斯斯咳嗽膠囊後，尿液則檢測出超出陽性反應閾值 10μg/ml 之濃度，且在 30 小時時其濃度仍高於閾值。由實驗結果可知，運動員在比賽期間應避免服用含甲基麻黃鹼之感冒藥，即使真的必須服用此類藥物來解除感冒症狀，也必須選擇單次劑量含量在 14mg 以下，運動員若在比賽前 30 個小時間，服用含 14mg 以上甲基麻黃鹼之感冒藥，則尿液呈陽性反應之機率是相當高的。

關鍵詞：甲基麻黃鹼，高效液相層析，感冒藥

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