HIGH PERFORMANCE LIQUID CHROMATOGRAPHIC DETERMINATION OF DEXTROMETHORPHAN AND ITS METABOLITE IN HUMAN URINE

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ABSTRACT A reverse-phase high-performance liquid chromatographic method was established for the determination of dextromethorphan (DM) and its metabolite dextrophan (DX) in human urine. The sample was purified by an acid-base extraction followed by HPLC analysis on a phenyl column. The eluate was monitored by fluorescence at 280 nm (excitation) and 310 nm (emission). The limit of detection of DM was 0.023 mg•L^{-1}. The within-day and between-day RSD% of DM and DX were all lower than 5%. The average recovery was 104.78%.

KEY WORDS High-performance liquid chromatography; Dextromethorphan; Dextrophan

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Genetic differences in certain drug metabolizing enzymes such as debrisoquin/sparteine 4-hydroxylase (CYP2D6)[1,2] have been shown to be associated with risk for developing adverse drug reaction. The CYP2D6 polymorphism has the most important influence because this enzyme also metabolizes a number of other drugs. The diminished oxidative metabolism of these drugs in poor metabolizers (PMs) can lead to high concentrations and toxic effects. However, in some extensive metabolizers (EMs) there will be minimal efficacy at low concentrations.

Previous studies[3,4] demonstrated that the polymorphism in dextromethorphan *O*-demethylation cosegregates with that in debrisoquin hydroxylation. This has led to use DM, an over-the-counter cough preparations, as a probe drug for CYP2D6 polymorphic screening recently[5,6]. However, these methods are either semiquantitative[5] or not sensitive enough[6] to discriminate possible intermediate metabolizers (IMs) from EMs accurately.

The present work reports a reverse-phase high-performance liquid chromatographic (HPLC) determinations of DM and its metabolite DX in human urine. The method can detect DM at 0.023 mg•L^{-1}.

MATERIALS AND METHODS

Chemicals and materials DM and DX were provided by Hoffman-La Roche Inc. (USA). Heptane sulfonic acid and β-glucuronidase (Type H-3, from Helix prematia, 125,000 u•ml^{-1}) were purchased from Sigma (USA). Water was doubly distilled. All other solvents and chemicals were of reagent grade.

Apparatus The HPLC system consisted of a Beckman 114M pump, a K501 6-valve injector, a Shimadzu RF-10A fluorescence detector and a Shimadzu C-R1B integrator. A

mobile phase of acetonitrile: water containing 10 mmol·L^{-1} KH$_2$PO$_4$ and 10 mmol·L^{-1} hexane sulfonic acid (35 : 65, v/v, pH 4.0) was pumped at 1.2 ml·min^{-1} through a Zorbax phenyl (4.6 mm×25 cm, 5 μm) column. The detector was set for excitation at 280 nm and emission at 310 nm.

Preparation of standards and controls of DM and DX A stock solution of DM (100.0 mg·L^{-1}) and DX (1000.0 mg·L^{-1}) was prepared in 0.01 mol·L^{-1} HCl and stored at 4℃. Urine standards were prepared by serial dilution of the stock solution with blank urine over concentration ranges of 0.025 ~ 1.60 mg·L^{-1} of DM and 0.50 ~ 32.0 mg·L^{-1} of DX. The low and high urine controls for DM were 0.15 mg·L^{-1} and 1.0 mg·L^{-1}, respectively. The low and high urine controls for DX were 1.5 mg·L^{-1} and 10.0 mg·L^{-1}, respectively.

Assay procedure To a 1.0 ml urine sample in a plastic culture tube were added 20 μl of β-glucuronidase and 1.0 ml 0.1 mol·L^{-1} acetate buffer (pH 5.0). The sample was vortexed and incubated at 37℃ for 18 h. After incubation, the sample was treated with 0.1 ml of 3 mol·L^{-1} NaOH and extracted with 5 ml of hexane—n-butanol (90:10, v/v). The organic layer was transferred to another culture tube and back-extracted into 200 μl of 0.01 mol·L^{-1} HCl. A 20 μl portion of the aqueous phase was injected onto the HPLC system.

RESULTS

1　Chromatograms of DM and DX

Figure 1 shows sample HPLC chromatograms of DM and DX from urines of typical EM and PM subjecs. Good resolutions have been achieved by our HPLC conditions. All compounds of interest were resolved to baseline. No interference peak was found near DM and DX, which were eluted around 9 min and 6 min respectively.
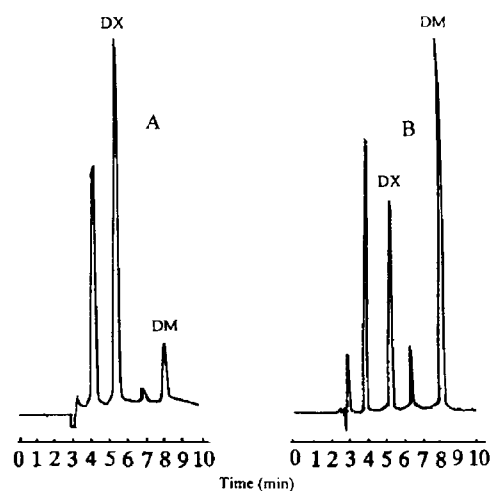

Fig 1　Chromatograms of dextromethorphan (DM) and dextrophan (DX) in urine from an extensive metabolizer subject (A) and a poor metabolizer subject (B). HPLC conditions are given in the text.

2　Linearity and detection limit

Standard curves of DM and DX were constructed by plotting peak area versus concentration and were linear over a 64-fold concentration range for both DM (Y = 574969X −6707, γ = 0.9998) and DX (Y = 9767X − 1280, γ = 0.9995). External standard method was used to calculate sample concentrations of subjects. Based on a signal-to-noise ratio of 5:1, the detection limits for DM and DX were 0.023 mg·L^{-1} and 0.031 mg·L^{-1}, respectively.

3　Reproducibility and recovery

The within-day relative standard deviations (RSD) for five DM low and high controls were 3.69% and 2.68%, respectively. The within-day RSDs for five DX low and high controls were 1.90% and 2.60%, respectively. The between-day RSDs for five DM and DX low and high controls were 3.42%, 2.09% and 4.63%,

4.80％，respectively（Table 1）. The mean recovery was 105.34％ for DM and 104.47％ for DX.

Tab 1　Reproducibility and recovery of DM and DX in urine（$n = 5$）

		Added (mg•L^{-1})	Within-day			Between-day			Recovery（%）
			$\bar{x}$	s	RSD（%）	$\bar{x}$	s	RSD（%）	
DM	L	0.15	0.16	0.006	3.69	0.16	0.006	3.42	106.67
	H	1.00	1.04	0.028	2.68	1.03	0.021	2.09	104.00
DX	L	1.50	1.57	0.029	1.90	1.50	0.072	4.63	103.73
	H	10.00	10.52	0.278	2.60	9.90	0.475	4.80	105.20

DM: Dextromethorphan; DX: Dextrophan

4　Extraction efficiency

Table 2 depicts the extraction efficiencies of DM and DX from urine. By comparing the concentration of extracted urine samples with that of unextracted samples containing specific DM（0.30，0.60 and 1.20 mg•L^{-1}）and DX（3.0，6.0 and 12.0 mg•L^{-1}），the extraction efficiency was determined to be 66.67％～81.00％ for DM and 88.67％～93.50％ for DX.

Tab 2　Extraction efficiency（%）of DM and DX in urine

No.	DM (mg•L^{-1})			DX (mg•L^{-1})		
	Added	Found	%	Added	Found	%
1	0.30	0.20	66.67	3.0	2.66	88.67
2	0.60	0.40	66.67	6.0	5.61	93.50
3	1.20	0.97	81.00	12.0	10.95	91.25

DISCUSSION

Previous HPLC method for determination of DM and DX used thebaine[5] as the internal standard. But，thebaine showed no fluorescence response under 280 nm excitation and is unavailable as it is a controlled substance. According to our experiment，the peak area response was linear over the concentration ranges of the DM（$\gamma = 0.9998$）and DX（$\gamma = 0.9995$）standards. The variability of the slope（RSD）of the calibration curve over a 4-week period（$n =$ 4）was 4.23％ for DM and 7.64％ for DX. Hence，determinations of DM and DX in our study were achieved by using a calibration curve without an internal standard.

The within-day and between-day RSD for low and high control samples of DM and DX were all below 5％. The average recovery was 104.90％. The extraction efficiency of the assay is satisfactory. In addition，the simple acid-base extraction step is more convenient and cheaper than other assays which require solid phase extraction minicolumns[5].

For most urine samples from subject who took DM for CYP2D6 polymorphic screening，the DX levels were high enough for analysis. However，the DM levels were often very low as a result of major EMs in Chinese. Thus，it is very important to have a higher sensitivity of HPLC to discriminate possible IMs from EMs. With the use of fluorescence detection，the sensitivity of DM from UV detection of 0.11～0.21 mg•L^{-1}[6] was increased to 0.023 mg•L^{-1}. The increased sensitivity would be helpful in phenotyping individuals with extremely rapid metabolism of dextromethorphan.

In conclusion，the HPLC determination of DM and DX has a higher sensitivity than that utilizing UV detection. It is very important for more accurate screening of CYP2D6 polymorphism.

With the simplification of extraction procedure and calibration without an internal standard, we could determine concentrations of DM and DX more conveniently and cheaper in clinical setting.

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人尿中右美沙芬及其代谢物的高效液相色谱测定法

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摘要　用反相高效液相色谱法测定人尿中右美沙芬(DM)及其代谢物去甲右美沙芬。尿样经酸、碱提取纯化,直接进样 HPLC 系统。样品经苯基柱分离后在荧光(激发波长 280 nm,发射波长 310 nm)下检测,以乙腈—含 10 mmol·L^{-1} KH$_2$PO$_4$,10 mmol·L^{-1}庚磺酸盐水溶液(35:65,pH 4.0)为流动相,外标法定量。DM 的最低检测浓度为 0.023 mg·L^{-1}。DM 和 DX 的日内和日间相对标准差均小于 5%。平均回收率为 104.90%。本法为临床筛选异喹胍羟化酶活性与合理用药提供了一种简便、安全的方法。

关键词　高效液相色谱法;右美沙芬;去甲右美沙芬

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