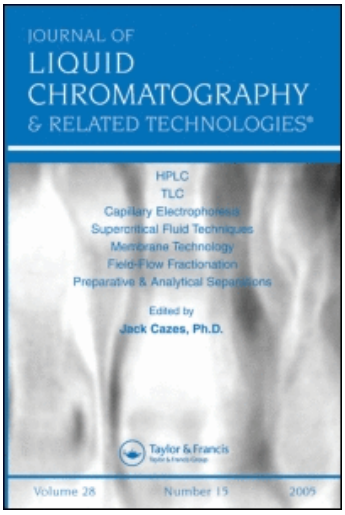

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DETERMINATION OF PHENYTOIN AND ITS MAJOR METABOLITE, 5-(p-HYDROXYPHENYL)-5-PHENYLHYDANTOIN IN URINE BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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ABSTRACT

Phenytoin is used in the prevention and treatment of seizures. It is important to measure the concentrations of both phenytoin and its major metabolite, 5-(p-hydroxyphenyl)-5-phenylhydantoin (p-HPPH) to fully describe the pharmacokinetics of phenytoin in patients. The method utilized a Water 10 μm μBondapak C-18 column, a mobile phase containing 45% acetonitrile and 55% distilled water, and a detection wave length of 230 nm. The retention times of p-HPPH, and phenytoin, and internal standard, 5-(4-methylphenyl)-5-phenyl-hydantoin (TPH) were 4.2, 6.4, and 8.1, minutes respectively. The correlation coefficients for p-HPPH and phenytoin were 0.999 for both compounds. The accuracy of the method ranged from 98.8 to 108.8% for p-HPPH and from 94.3 to 102.1% for phenytoin at concentrations ranging from 0.05 to 0.35 mg/mL for each compound. The coefficient of variation ranged from 0.1 to 9.6% for p-HPPH and 0.8 to 5.0% for phenytoin. This method was successfully used to quantify phenytoin and p-HPPH in the urine of critically ill infants and children.

INTRODUCTION

Phenytoin is commonly used in the prevention and treatment of seizures. The pharmacokinetics of phenytoin is nonlinear and is altered in conditions such as head injury, febrile illnesses, and renal failure. Thus multiple studies designed to characterize the disposition of phenytoin and its metabolites have been undertaken.

A variety of methods for the determination of the urinary excretion of phenytoin and p-HPPH have been described (1-4). Our method offers a number of advantages over the other reported methods: 1) it is associated with lower cost; 2) it does not require a column heater; and, 3) it requires less time for analysis of phenytoin and p-HPPH.

The purpose of this article is to describe a simple, reproducible high-performance liquid chromatography (HPLC) method for the determination of phenytoin and the major metabolite, 5-(p-hydroxyphenyl)-5-phenylhydantoin (p-HPPH), in urine. The method was successfully used to characterize the pharmacokinetics of phenytoin in critically ill pediatric patients.

Equipment

HPLC instrumentation included Hewlett Packard 1050 series pump, autosampler, variable wave length

detector, and Hewlett Packard 3396A integrator. A Waters 10 μm Bondapak C-18 column (Millipore Corporation, Milford, MA) was used. Additional equipment included a digital pH meter (Orion, model 701A), Organomation N-Evap nitrogen evaporator, Lab-Line Instruments water bath, and International Equipment Company Centra-4B centrifuge.

Chemicals and Reagents

The chemicals and reagents used included sodium acetate anhydrous (Mallinckrodt, lot number 7372KASG), methanol (Baxter, lot number BC386), acetonitrile (Baxter, lot number BB207), 5,5-diphenylhydantoin (phenytoin; Aldrich, lot number 57818TM), DL-5-(p-hydroxyphenyl)-5-phenylhydantoin (Aldrich, lot number HJ3702HE), 5-(4-methylphenyl)-5-phenyl-hydantoin (TPH) (Aldrich, lot number 05513BX HX), beta-glucuronidase (Sigma, lot number 11OH6812), glacial acetic acid (Mallinckrodt, lot number 2504KAPH-A), buffer solution pH 7.0 (Fisher, lot number 01090-24), and buffer solution pH 4.0 (Fisher, lot number 910043-24).

Mobile Phase

The mobile phase consisted of 45% acetonitrile and 55% distilled water. The mobile phase was filtered through a 0.45 μm nylon 66 filter, and degassed with helium.

Sample Preparation

Standard urine samples between 0.05 and 0.35 mg/mL of p-HPPH and phenytoin were prepared and handled exactly like patient samples. Ten micrograms of internal standard, 5-(4-methylphenyl)-5-phenyl-hydantoin (TPH), were placed in a tapered polypropylene conical centrifuge tube and evaporated to dryness under nitrogen. A 200 μL aliquot of urine (standards or patient samples), 200 μL of sodium acetate buffer (0.1M, pH 5) and 1000 U/0.1 mL of beta-glucuronidase were added and incubated in a water bath at 37°C overnight. Ethyl acetate, 5 mL, was added to the tube and the tubes were centrifuged at 2500 g for at least 2 minutes. Four milliliters of the upper, organic phase were transferred to a second polypropylene tube and evaporated to dryness under nitrogen. The residue was reconstituted with 100 μL of methanol. The samples were transferred to autosampler vials for analysis.

Chromatographic Conditions

The flow rate was 1.0 mL/minute. The detector was set at a wave length of 230 nm. Ten microliters were injected onto the HPLC column. All measurements were performed at room temperature.

RESULTS AND DISCUSSION

Each chromatographic run required approximately 10 minutes. The total p-HPPH (conjugated plus

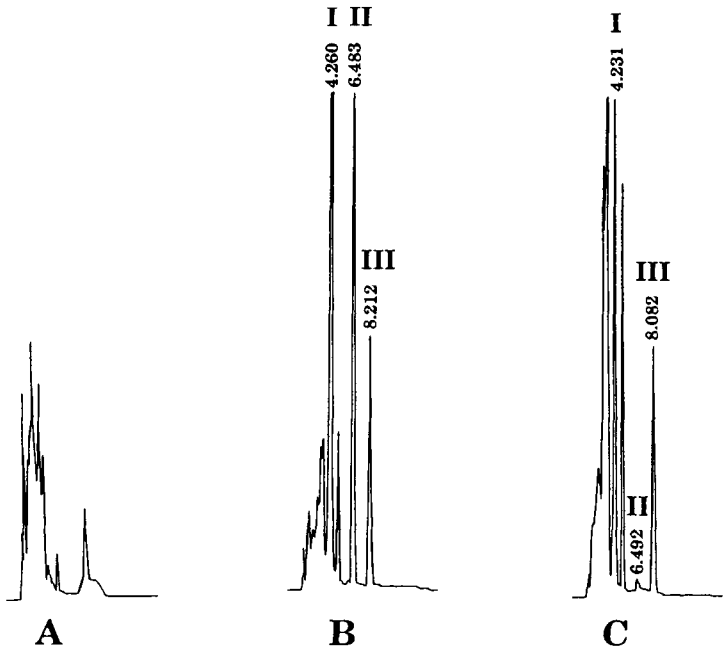

Figure 1. Chromatographs showing
A: Blank urine;
B: Typical standard containing p-HPPH (I), phenytoin (II), and TPH (internal standard, III);
C: Patient sample containing p-HPPH (I), phenytoin (II), and TPH (III).

unconjugated), phenytoin, and TPH eluted at approximately 4.2, 6.4, and 8.1 minutes, respectively. Typical chromatograms of blank urine, spiked urine, and a patient sample are shown in Figure 1.

Linearity of the concentration range was determined by linear regression analysis of peak height ratios of p-HPPH/TPH and phenytoin/TPH as shown in Table 1.

TABLE 1.

Peak Height Ratios of p-HPPH:TPH and Phenytoin:TPH at Various Concentrations.

p-HPPH concentration, mg/mL	Peak height ratio
0.05	2.525
0.10	4.779
0.15	7.044
0.20	9.232
0.25	11.24
0.30	13.45
0.35	15.47

Phenytoin concentration, mg/mL	Peak height ratio
0.005	0.0817
0.05	0.627
0.10	1.358
0.15	2.099
0.20	2.796
0.25	3.495
0.30	4.107
0.35	4.746

The correlation coefficients (r) for p-HPPH and phenytoin were 0.999 for both compounds. The accuracy of the method ranged from 98.8 to 108.8% for p-HPPH and from 94.3 to 102.1% for phenytoin at concentrations ranging from 0.05 to 0.35 mg/mL for each compound. The method overestimates phenytoin concentrations by 24% at a concentration of 0.005 mg/mL. The coefficient of variation ranged from 0.1 to 9.6% for p-HPPH and from 0.8 to 5.0% for phenytoin. (Table 2)

This method was used to determine the pharmacokinetics of phenytoin in pediatric patients following head injury. A patient with head injury

TABLE 2.

Accuracy of p-HPPH and Phenytoin Measurements.

Known concentration, mg/mL	Concentration found, (mean + SD), mg/mL	% of known concentration found
p-HPPH		
0.05	0.052 ± 0.005	103.6
0.10	0.106 ± 0.009	106.2
0.15	0.163 ± 0.012	108.8
0.20	0.214 ± 0.015	107.2
0.25	0.267 ± 0.021	106.8
0.30	0.308 ± 0.013	102.7
0.35	0.346 ± 0.014	98.75
Phenytoin		
0.005	0.006 ± 0.0003	124.2
0.05	0.047 ± 0.00006	94.3
0.10	0.099 ± 0.001	99.2
0.15	0.153 ± 0.002	102.1
0.20	0.204 ± 0.002	102.0
0.25	0.255 ± 0.002	102.0
0.30	0.300 ± 0.005	99.8
0.35	0.346 ± 0.006	98.9

received phenytoin 10.5 mg/kg/day. The concentrations of phenytoin and p-HPPH in the urine was 0.01 mg/mL and 0.11 mg/mL, respectively. The amount of phenytoin and p-HPPH as a percentage of administered phenytoin dose was 75% and 6.7%, respectively.

This proved to be a simple, reproducible HPLC method for determining the amount of phenytoin and p-HPPH in urine.

REFERENCES

1. Sawchuk, R. J., Cartier, L. L., Liquid-chromatographic Method for Simultaneous

Determination of Phenytoin and 5-(4-hydroxyphenyl)-5-phenylhydantoin in Plasma and Urine, Clin Chem., 26/27, 835-839, 1980.

2. Dickinson R. G., Hooper, W. D., Patterson, M., Eddie, M. J., Maguire, B., Extent of Urinary Excretion of p-hydroxyphenytoin in Healthy Subjects Given Phenytoin, Ther. Drug Monitor., 7, 283-289, 1985.

3. Boucher, B.A., Rodman, J. H., Jaresko, G. S., Rasmussen, S. N., Watridge, C. B., Fabian, T. C., Phenytoin Pharmacokinetics in Critically Ill Trauma Patients, Clin. Pharmacol. Ther., 44, 675-683, 1988.

4. Nolan, P.E., Marcus, F.I., Hoyer, G.L., Bliss, M., Gear, K., Pharmacokinetic Interaction Between Phenytoin and Amiodarone in Healthy Volunteers, Clin. Pharmacol. Ther., 46, 43-50, 1989.

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