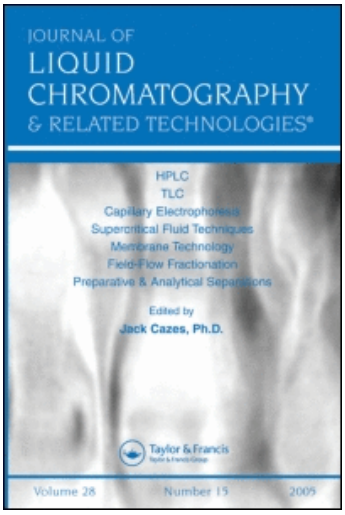

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HPLC DETERMINATION OF 2-PHENYL-4-QUINOLONE IN RABBIT SERUM

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ABSTRACT

A specific and sensitive high-performance liquid chromatographic method was developed for the assay of 2-phenyl-4-quinolone (YT-1) in rabbit serum. The proposed method is simple, rapid (deproteinization time, within 5 min; analysis time, ca. 20 min), sensitive [detection limit, ca. 0.05 μg/mL serum (at a signal to noise ratio of 3:1)], highly selective, and reproducible [relative standard deviation, ca. 1.3% ($n = 3$)]. The calibration graph for YT-1 was linear ($r = 0.998$) in the range of 0.1–100.0 μg/mL. The recovery of YT-1 was greater than 96% by the standard addition method. This validated method is available for application to pharmacokinetic studies of YT-1.

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INTRODUCTION

2-Phenyl-4-quinolone (YT-1), a newly synthesized compound, shows potent cytotoxicity against many kinds of carcinoma (1). Its cytotoxic activities on lung carcinoma (A-549), ileocecal carcinoma (HCT-8), epidermoid carcinoma of the nasopharynx (KB), two murine leukemia lines (P-388 and L1210) (1) breast cancer (MCF-7), renal cancer (CAKI-1), and melanoma (RPMI-7951, SKMEL-2) (1,2) had been reported previously. In addition, it was also shown that YT-1 had a positive inotropic effect (3): YT-1 inhibited the neutrophil respiratory burst and local edema formation (4,5) and aspirin-like antiplatelet activity (6,7).

These results stimulated us to develop YT-1 as a new cytotoxic agent for clinical use. Many preclinical studies, such as pharmacokinetics, pharmacodynamics, and toxicity tests, are important for the development of a new drug. A high-performance liquid chromatography (HPLC) method for the detection of YT-1 in rabbit serum would be helpful for the pharmacokinetic study. But, no HPLC method had been described before. Therefore, in this study an HPLC method for assay of YT-1 was developed.

EXPERIMENTAL

Apparatus

A Shimadzu LC-10AT with SPD-M10AVP detector with detection wavelength at 254 nm was used for the determination of YT-1 in rabbit serum. A reversed-phase column (Lichrosher 100RP-18, 5 μm, 250 $\times$ 4 mm end-capped Merck 50995) was used to separate the rabbit serum excipients.

Reagents and Chemicals

YT-1 was synthesized according to the process by Staskun and Israelstam (8) and was purified three times by recrystallization with alcohol. The purity of YT-1 was greater than 98% by the HPLC assay method with photodiode array detection. Butylparaben was obtained from E. Merck, acetonitrile was purchased from TEDIA, and β-glucuronidase was purchased from Sigma. Other reagents or solvents used were of analytical reagent grade or HPLC grade.

Preparation of Standard Solutions

The stock solution was prepared by dissolving 100 mg of YT-1 with alcohol to 100 mL. Certain amounts of YT-1 from the stock solution were taken and diluted

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to make 0.1–100.0 μg/mL working standard solutions separately with alcohol, and then spiked into 50 μL of rabbit serum.

Serum proteins were precipitated by adding 0.3 mL of acetonitrile and removed by centrifugation (9,10). The supernatant (300 μL) was assayed by the validated HPLC method with 20 μL of butylparaben as an internal standard.

Rabbit Experiment

Six male New Zealand white rabbits, which weighed about 2–3 kg, were used in preliminary pharmacokinetic studies. Before single intravenous (i.v.) and by mouth (p.o.) administrations of 10 mg/kg of YT-1, the rabbits were fasted overnight with water adlibitum. Blood samples (1.0 mL) were collected periodically at intervals from the marginal vein of the ear up to 360 and 600 min after i.v. and p.o. administration. The serum was separated after centrifugation at 5000*g* for 5 min and stored at -20°C until analysis.

Preparation of Serum Samples

Samples of each serum (50 μL) were introduced into 50 μL of β-glucuronidase separately. We mixed them on a vortex for 30 s and incubated them at 37°C for 2 h. After incubation a cleanup procedure of acetonitrile (0.3 mL) containing 2.4 μg/mL of butylparaben as an internal standard was performed on each sample preparation. We mixed the samples on a vortex again for 30 s and then centrifuged them for 5 min at 3000*g*.

Another 50 μL of each serum sample was treated with the same cleanup procedure without added β-glucuronidase. For both kinds of treated serum samples, 20 μL of each treated supernatant was then assayed by HPLC directly.

HPLC Determination

The gradient elution system comprised two phases: phase A (water) and phase B (acetonitrile). The linear profile adopted was gradient: $t = 0$, %B = 45; gradient $t = 10$ min, %B = 60; and gradient $t = 16$ min, %B = 45. Butylparaben was used as an internal standard. The flow rate was 0.7 mL/min. After each run, the mobile phase was allowed to maintain the initial condition for 4 min for stabilization.

Nine prepared standard solutions of YT-1 concentrations in the range of 0.1–100.0 μg/mL were available for the establishment of a calibration curve, and butylparaben was used as an internal standard. The peak area ratios of YT-1

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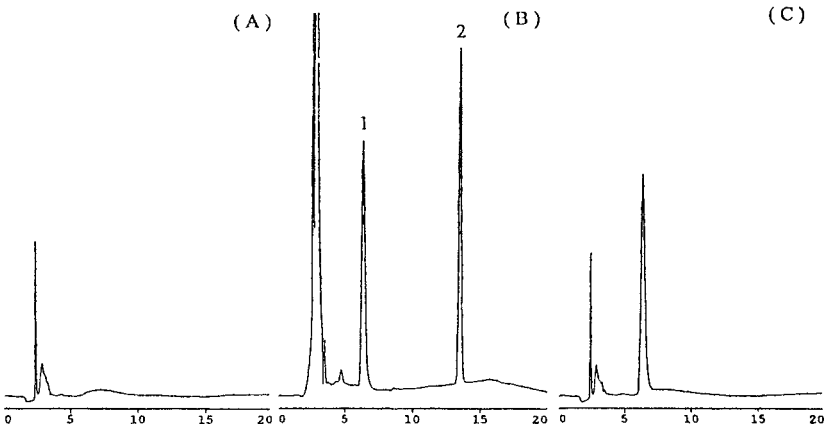

Figure 1. Typical chromatograms of (A) drug-free serum, (B) a serum sample taken 90 min after p.o. administration of 10 mg/kg of YT-1 to rabbit, and (C) a serum spiked with YT-1. Peaks: 1) YT-1; 2) internal standard.

to butylparaben were determined. The serum samples from rabbits were assayed under the same conditions, and the concentration of YT-1 validation in serum was then calculated. The intraday and interday accuracy and precision of the method were validated; the recovery of YT-1 from serum was also determined.

Table 1. Experimental Values Obtained in the Recovery Test for 2-Phenyl-4-Quinolone by HPLC[a]

Spiked Conc. (μg/mL)	A[b]	B[b]	Recovery (%)[c]
0.1	0.0008	0.0008	100.0
0.2	0.0017	0.0017	100.0
1	0.0093	0.0094	98.9
2.5	0.0211	0.0214	98.6
5	0.0440	0.0446	98.7
10	0.0847	0.0858	98.7
25	0.2120	0.2137	99.2
50	0.4089	0.4230	96.7
100	0.8577	0.8705	98.5

[a] A, when blank serum was used as the solvent; B, when an aqueous solution was used as the solvent.
[b] Mean of 2-phenyl-4-quinolone/butylparaben response ration ($n = 3$).
[c] (A/B) $\times$ 100%.

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Table 2. Interday and Intraday Precision for Analysis of 2-Phenyl-4-Quinolone in Rabbit Serum

Spiked Conc. (μg/mL)	Mean[a]	Interday SD	CV (%)[b]	Mean[a]	Intraday SD	CV (%)[b]
0.1	0.113	0.002	1.35	0.110	0.002	1.38
0.2	0.217	0.003	1.16	0.211	0.003	1.19
1	1.001	0.016	1.55	0.986	0.009	0.86
2.5	2.493	0.019	0.75	2.484	0.006	0.25
5	5.078	0.064	1.26	4.987	0.007	0.14
10	9.950	0.088	0.88	10.005	0.022	0.22
25	24.959	0.090	0.36	24.993	0.011	0.04
50	48.095	0.126	0.26	48.538	0.405	0.83
100	100.490	0.863	0.86	100.066	0.071	0.07
R^2=		0.9996			0.9998	

[a] $N = 3$.
[b] CV, coefficient of variation.

RESULTS AND DISCUSSION

The retention times of the internal standard (butylparaben) and YT-1 were 13.6 and 6.3 min, respectively. No interfering peak was found in the chromatogram (Fig. 1). YT-1 was shown to be stable during all procedures. The calibration curve

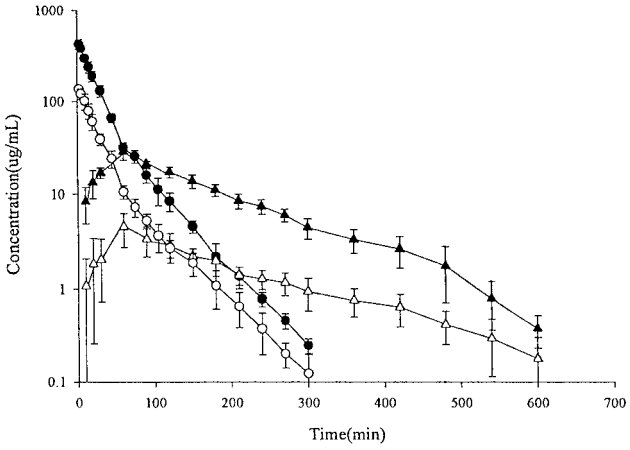

Figure 2. Serum concentration-time profiles of YT-1 after 10 mg/kg YT-1 i.v. and β-glucuronidase (closed circles), no YT-1 and β-glucuronidase (open circles), 10 mg/kg YT-1 p.o. and β-glucuronidase (closed triangles), and no YT-1 and β-glucuronidase (open triangles) in crossover administration to six rabbits. Results are shown as mean ± SD.

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was linear over the working concentration of YT-1 in a range of 0.1–100.0 μg/mL with a correlation coefficient greater than 0.9998. The limit of detection in serum was 0.05 μg/mL. The recovery of YT-1 from the serum was greater than 96%. Both the coefficients of variation of the intraday and interday were less than 2% as shown in Tables 1 and 2.

The method was then applied to the pharmacokinetic study in rabbit, after administration of YT-1 through the oral or intravenous route. Typical serum concentration-time profiles are shown in Figure 2.

It is shown that for the serum treated with β-glucuronidase, the concentrations of YT-1 calculated were higher than those for the other serum without treatment with β-glucuronidase. From i.v. and p.o. administration, it appears that most of the YT-1 existing in serum was the metabolites. The serum sample treated with β-glucuronidase might reverse the metabolites back to the free form YT-1 and increase the detected results.

CONCLUSION

In the present study we developed a rapid and precise method for the determination of YT-1 in rabbit serum by HPLC. The method demonstrated acceptable linearity, sensitivity, and accuracy.

No matter which administration route of YT-1 to rabbits was used, the concentrations of YT-1 in the sample serum could be detected accurately. This result indicated that the proposed method could also be recommended for a YT-1 pharmacokinetic study.

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