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Determination of Ondansetron and Metabolites in Plasma and its Pharmacokinetics in Cancer Patients

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ABSTRACT

Ondansetron is a novel 5-hydroxy-tryptamine$_3$ (5-HT$_3$) receptor antagonist which is a drug used clinically for chemotherapy-, radiotherapy-and postoperation induced nausea and emesis. A simple, sensitive, precise and accurate HPLC method for the detemination of ondansetron and its metabolites 7-hydroxy-ondansetron and 8-hydroxy-ondansetron in plasma is reported. Samples were prepared by a traditional, inexpensive solvent extraction method. The method employed a CN analytical column with an isocratic mobile phase of 20% acetonitrile in 80% 0.0125 M NH_4OAc (pH = 4.7) and ultraviolet detection at 305 nm. Using this method, the calibration curves of ondansetron, 7-hydroxy-ondansetron and 8-hydroxy-ondansetron in plasma in the range of interest (20-800 ng/ml, 100-800 ng/ml and 100-1000 ng/ml, respectively) showed good linearity ($r > 0.999$) and precision (both between-day and within-day coefficients of variation less or around 10%). The lower detection limits of this assay method for ondansetron and its 7-OH and 8-OH metabolites were 5 ng/ml, 10 ng/ml and 10 ng/ml respectively. This method has been successfully applied to the pharmacokinetic study of ondansetron in cancer patients receiving single i.v. infusion followed by a multiple oral dose of ondansetron.

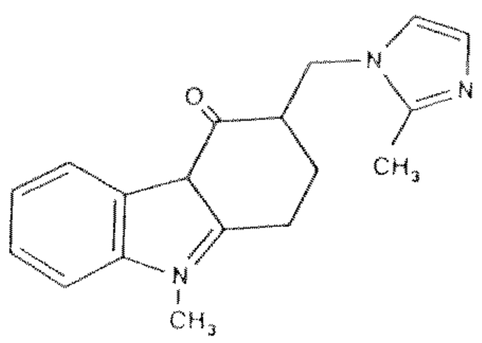
Key word: Ondansetron, assay method, pharmacokinetics, cancer patients.

INTRODUCTION

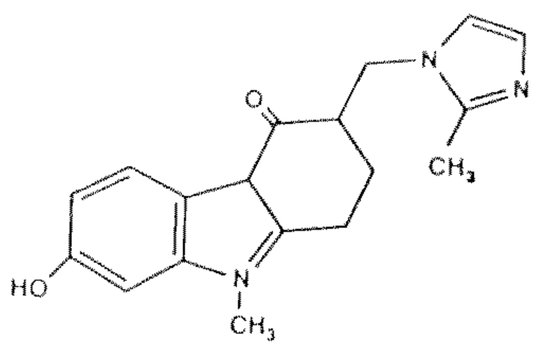
Ondansetron (1, 2, 3, 9-tetrahydro-9-methyl-3-[(2-methyl-1H-imidazol-1-yl) methyl]-4H-carbazol-4-one hydrochloride dihydrate; (Fig. 1) is a novel antiemetics and antinauseant with the mechanism of being a potent 5-hydroxy-tryptamine$_3$ (5-HT$_3$) receptor antagonist. Ondansetron is currently used in clinical areas for radiotherapy-, chemotherapy[1]-and postoperation[2] induced nausea and emesis.

Ondansetron has been analyzed in plasma by high - pressure liquid chromatographu (HPLC)[3] and high-performance thin-layer chromatography (HPTLC)[4].

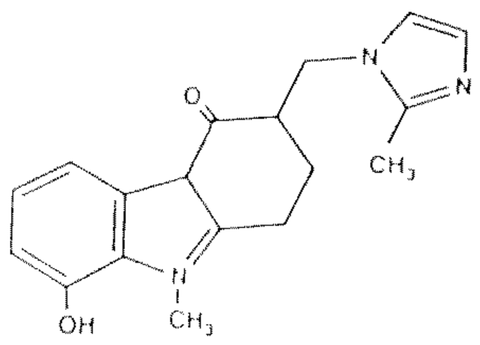
The HPLC method was sensitive, precise and accurate. However, an expensive solid phase extraction method using Bond Elute CN extraction cartridges was applied. The HPTLC method was also sensitive and accurate, whereas the equipment needed may not gener-



Ondansetron



7-hydroxy-ondansetron



8-hydroxy-ondansetron

Figure 1. Structures of Ondansetron, 7-hydroxy-ondansetron and 8-hydroxy-ondansetron

ally be available in other laboratories. This paper reports an HPLC method for the determination of ondansetron and its metabolites 7-hydroxy-ondansetron and 8-hydroxy-ondansetron in plasma. Samples were prepared by a traditional, inexpensive solvent extraction method with a rather simple preparation procedure and good lower detection limit for the pharmacokinetic study of ondansetron in cancer patients receiving single i. v. infusion followed by a multiple oral dose of ondansetron.

MATERIALS AND METHODS

Chemicals and Reagents

Ondansetron 7- and 8-hydroxy-ondansetron were kindly given by Glaxo Co. Taiwan. Daunorubicin as an internal standard was supplied by Farmitalia Carlo Erba (Taiwan branch). Acetonitrile, dichloromethane and n-hexane(Merck, Darmstadt F. R. G.) were HPLC grade used as mobile phase and extraction solvents. Ammonium acetate was purchased from Merck. Milli-Q water was prepared through a Milli-RO 60 water purification system (Millipore, Bedford, MA, U. S. A.).

Preparation of Standards

Ondansetron and metabolites were accurately weighed and dissolved in water to make 1 and 10 μg/ml stock solutions, except 8-hydroxy-ondansetron which was dissolved in 1:1 acetonitrile:water solution.

Calibration standards for ondansetron (20, 40, 80, 100, 200, 300, 400, 500 and 800 ng/ml), for 7-OH-ondansetron (100, 200, 300, 400, 500 and 800 ng/ml)and for 8-OH-ondansetron (100, 200, 300, 400, 500, 800 and 1000 ng/ml) were prepared by mixing 1 ml of blank plasma with an appropriate volume of ondansetron and metabolites' stock solutions. Daunorubicin (25 μg/ml in H_2O) was selected as an internal standard. The calibration curve in plasma was freshly constructed for each assay run during the studies.

Chromatography

HPLC was performed on a combined Waters and Kratos liquid chromatographic system with a Waters Model 712 WISP autosampler, a Waters Model 510 pump (Waters Assoc, Milford, MA, U. S. A.) and a ABI Model 783a variable-wavelength UV detector (Kratos, Ramsey, NJ, U. S. A.) set at 305 nm at a sensitivity of 0.005 a. u. f. s. A SIC schromatocorder 12 integrator (System Instruments, Tokyo, Japan) and a Waters column oven thermostated at 50 ℃ were also used. A self-packing CN analytical column (5μ, 250 X 4.6mm) and a guard column (7μ, 15 X 3.2mm) with

CN Brownlee™ packing material were also used. The packing material of the guard column was replaced every one or two weeks or when there was significant build-up of back-pressure.

Ondansetron, 7-, 8-hydroxy-ondansetron and the internal standard daunorubicin were eluded isocratically with acetonitrile: 0.0125 M NH_4OAc (pH = 4.7) (20: 80) as the mobile phase at a flow rate of 1.0 ml/min. Peak areas were integrated and recorded by the SIC-12 integrator. Mobile phase was always filtered through a 0.22 μm millipore filter followed by degassing.

Sample Preparation

The preparation consisted of a clean-up and extraction procedures. A 100 ul of internal standard, daunorubicin (25 μg/ml) was added to plasma which contained various or unknown ondansetron and its metabolites. The plasma sample (1 ml) was mixed with 0.5 ml of phosphate buffer (pH = 8.4) and followed by the addition of 10 ml of dichloromethane.

The first extraction procedure was done by mechanically rotating the sample for 60 min. After centrifugation (x 1020 g, 20 min) the lower organic layer was aspirated into another clean tube for evaporation. Filtered dry air was used for evaporation. A 300 μl of 0.0125 M NH_4OAc buffer (pH = 4.7) was added to the residue, mixed for 5 min, then another 2 ml of n-hexane was added and vortexed for 1 min for the clean-up extraction. The same centrifugation was performed for 5 min. The aqueous layer was removed and 200 μl was injected directly into the HPLC apparatus.

The overall extraction recoveries of ondansetron was studied. The extraction recovery (ER) was calculated form the following equation:

$$ER = \frac{PAp}{PAs} \times 100\%$$

where PAp is the peak area of ondansetron in plasma, and the PAs is the peak area of the equivalent amount of ondansetron in stock solution without extraction.

Assay Validation

To assess the precision and accuracy of this assay method, both within-day and between-day calibration curves (n = 6) with various concentrations of ondansetron and two metabolites (described in the section of Preparation of Standards) in plasma were constructed. The mean peak area ratio used throughout this study was calculated by dividing the sample peak

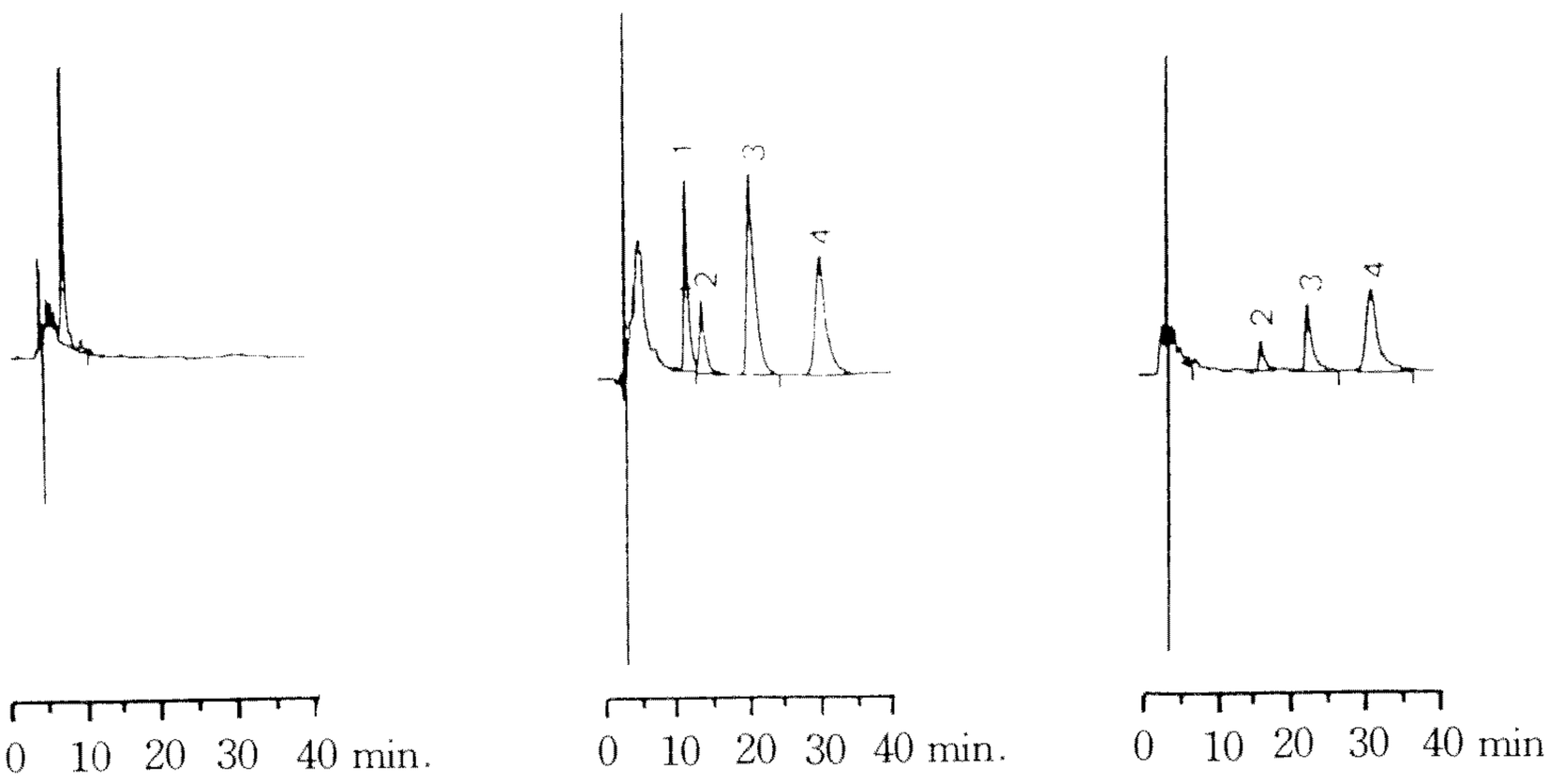


Figure 2. Chromatograms of (A) Blank Plasma, (B) Plasma Spiked with (1)7-hydroxy-ondansetron, (2)8-hydroxy-ondansetron, (3) Ondansetron and (4) Daunorubicin (Internal Standard) (C) Clinical Trial Sample Spiked with Daunorubicin from Cancer Patient Using CN Column at 305 um, the Ondansetron Plasma Concentration Was 23 ng/ml, the 8-hydroxy-metabolite Concentration Was 28 ng/ml.

area by the daunorubicin peak area in plasma.

Pharmacokinetic Study

Two adult patients with nasopharyngeal cancer received an ondansetron loading dose of 8 mg in 105 ml normal saline by i.v. infusion for 24 hrs. Another oral 8 mg ondansetron tablet t.i.d. was given on the second to the fifth day. Serial blood samples, namely 5 minutes before drug administration, 21, 21.5, 22, 22.5, 23, 23.5, and 24 hrs before the infusion stopped, and 0, 0.25, 0.5, 1, 1.5, 2, 4, 6, and 8 hrs after the last dose on the fifth day, were drawn. Blood samples obtained from the forearm vein with a heparin lock were collected. Plasma was separated immediately by centrifugation and was kept frozen (-60℃) until analysis.

RESULTS AND DISCUSSION

The highly sensitive and inexpensive sample extraction procedures described in this paper give a clear separation of the drug and its metabolites in blank and spike samples (Fig. 2). The lower detection limits for ondansetron, 7-OH-, 8-OH-ondansetron were 5 ng/ml, 10 ng/ml and 10 ng/ml respectively.

Accuracy was confirmed by determining the known concentration in plasma using calibration curves. The calibration curves of ondansetron, 7-OH- and 8-OH-ondansetron in plasma in the range of interest (20-800, 100-800 ng/ml, and 100-1000 ng/ml, respectively) showed good linearity ($r > 0.999$) and precision (both within-day and between-day coefficients of variation less or around 10%). (Tab. 1, 2, 3, and Fig. 3, 4, 5). The extraction recoveries of ondansetron were $98.01 \pm 5.06\%$ ($n=2$).

The method has been successfully applied to the pharmacokinetic study of ondansetron in cancer patients receiving single (8 mg/day) i.v. infusion as the loading dose followed by a t.i.d., multiple oral dose (8 mg tab.) of ondansetron. Both ondansetron and 8-hydroxy-ondansetron were detected. In one patient, ondansetron plasma levels were in the range of 20 ng/ml to 120 ng/ml, whereas, the 8-OH metabolite had a plasma range of 20 ng/ml to 160 ng/ml. (Fig. 6). This showed that 8-OH-ondansetron had a higher concentration than ondansetron and had an accumulation phenomenon at a steady state, since the 8-hydroxy-ondansetron concentration ratio of Cmax at first loading dose was much lower than the ratio at steady state (95:113). The other patient had no 8-OH-ondansetron for the entire week, and the ondansetron level was from 50 ng/ml to 210 ng/ml(Fig. 7). No 7-hydroxy-ondansetron was detected in these two patients. Also, from these two preliminary pharmacokinetic studies it is shown that ondansetron was absorbed very quickly with the time required to reach peak plasma concentration around 2 hrs.

Philip et al[3], Colthup et al[5], and Blackwell et al[6](Fig. 7), reported that the half-life of ondansetron in Caucacians was 3.1 hr, 3.2 ± 0.7 hr and 3.2 hr, respectively. Ondansetron has been on the market in Taiwan for over a year, and as yet there were no pharmacokinetic properties reported in Chinese patients. Large scale ondansetron pharmacokinetic studies were undertaken in Chinese cancer patients applying this analytical methodology.

We have reported a simple, precise and accurate HPLC method for the determination of ondansetron and its two metabolites, using an inexpensive liquid-liquid extraction method. This method had been applied to a pilot pharmacokinetic study in cancer patients.

ACKNOWLEDGEMENT

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Table 1. Between-day Standard Curve Data of Ondansetron in Plasma

Concentration	Ondansetron Peak Area Ratio		
(μg/ml)	Mean	S.D.	%CV
0.02	0.09	0.01	9.37
0.04	0.19	0.01	6.61
0.08	0.39	0.02	6.18
0.10	0.50	0.02	4.25
0.20	0.96	0.06	6.25
0.30	1.53	0.10	6.27
0.40	2.01	0.13	6.25
0.50	2.53	0.06	2.48
0.80	4.01	0.36	8.91

N = 6

Table 2. Between-day Standard Curve Data of 7-hydroxy-ondansetron in Plasma

Concentration	7-hydroxy-ondansetron Peak Area Ratio		
(μg/ml)	Mean	S.D.	%CV
0.10	0.27	0.02	6.13
0.20	0.50	0.04	7.80
0.30	0.75	0.03	4.63
0.40	1.05	0.07	7.14
0.50	1.28	0.04	3.17
0.80	2.01	0.13	6.29

N = 6

Table3. Within-day Standard Curve Data of 8-hydroxy-ondansetron in Plasma

Concentration	8-hydroxy-ondansetron Peak Area Ratio		
(μg/ml)	Mean	S.D.	%CV
0.10	0.04	0.00	8.85
0.20	0.20	0.01	5.67
0.30	0.12	0.02	1.39
0.40	0.66	0.05	3.06
0.50	0.82	0.03	2.10
0.80	1.49	0.07	1.66
1.00	1.86	0.10	0.84

N = 6

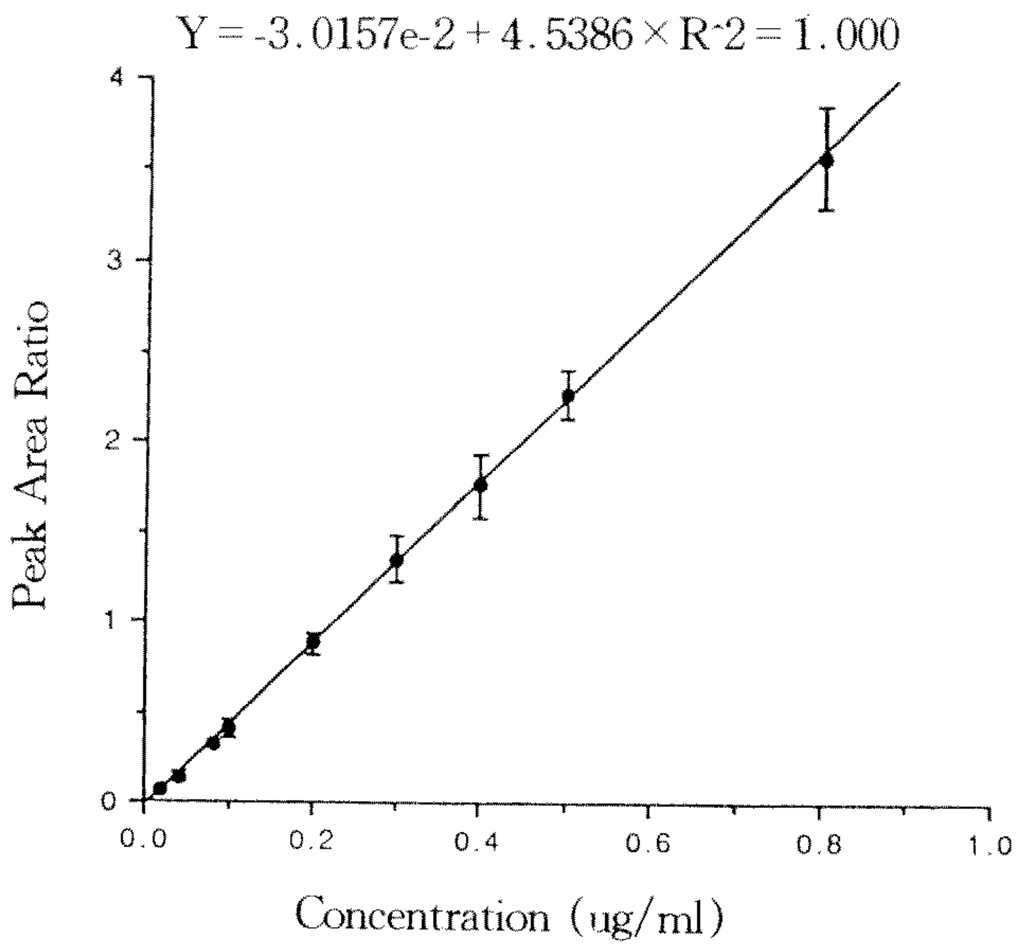

Figure 3. Within-day Standard Curve of Ondansetron in Plasma (Mean ± SD, N = 6)

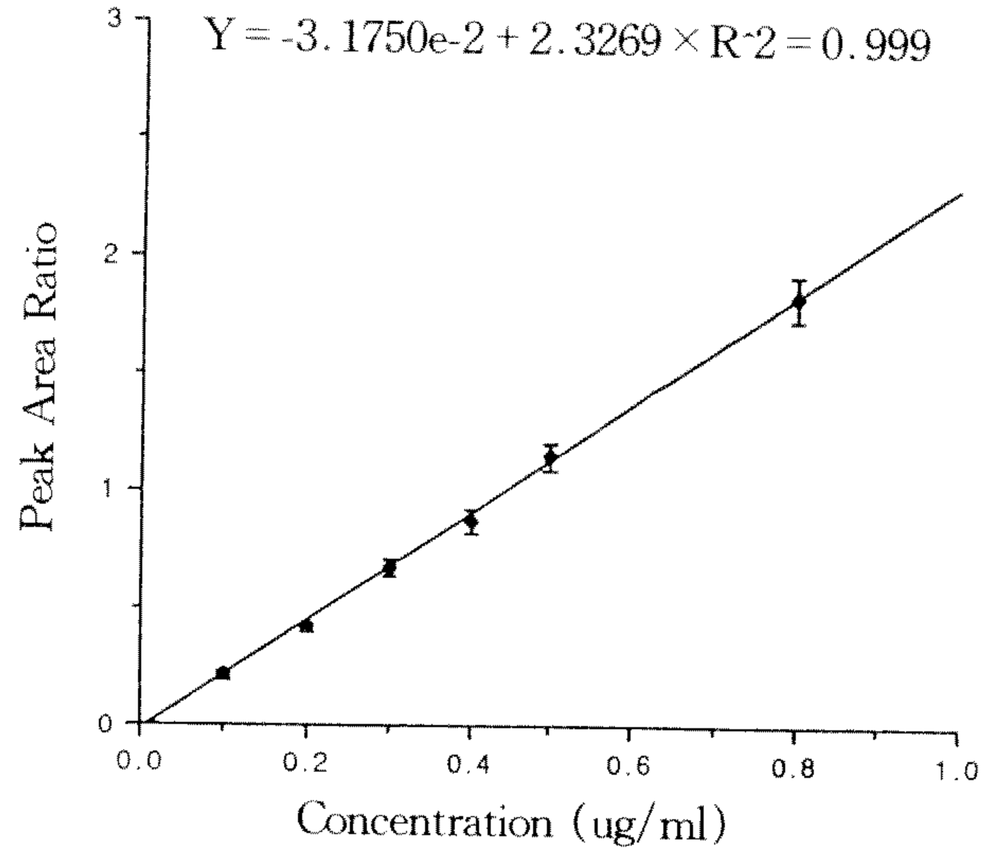

Figure 4. Within-day Standard Curve of 7-hydroxy-ondansetron in Plasma (Mean ± SD, N = 6)

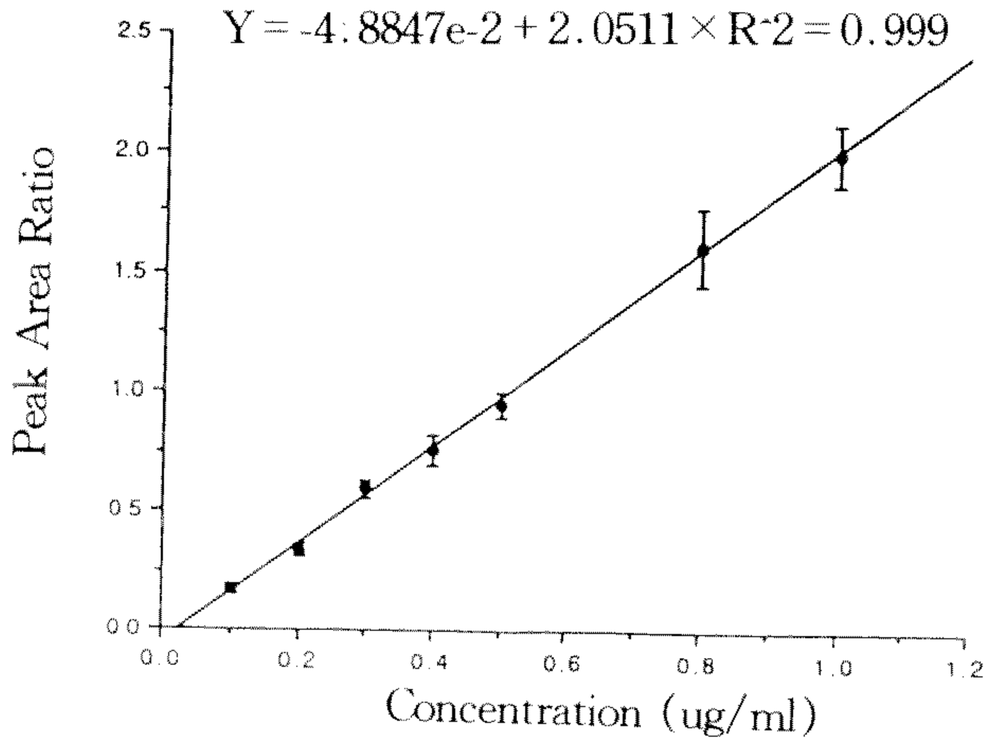

Figure 5. Between-day Standard Curve of 8-hydroxy -ondansetron in Plasma (Mean ± SD, N = 6)

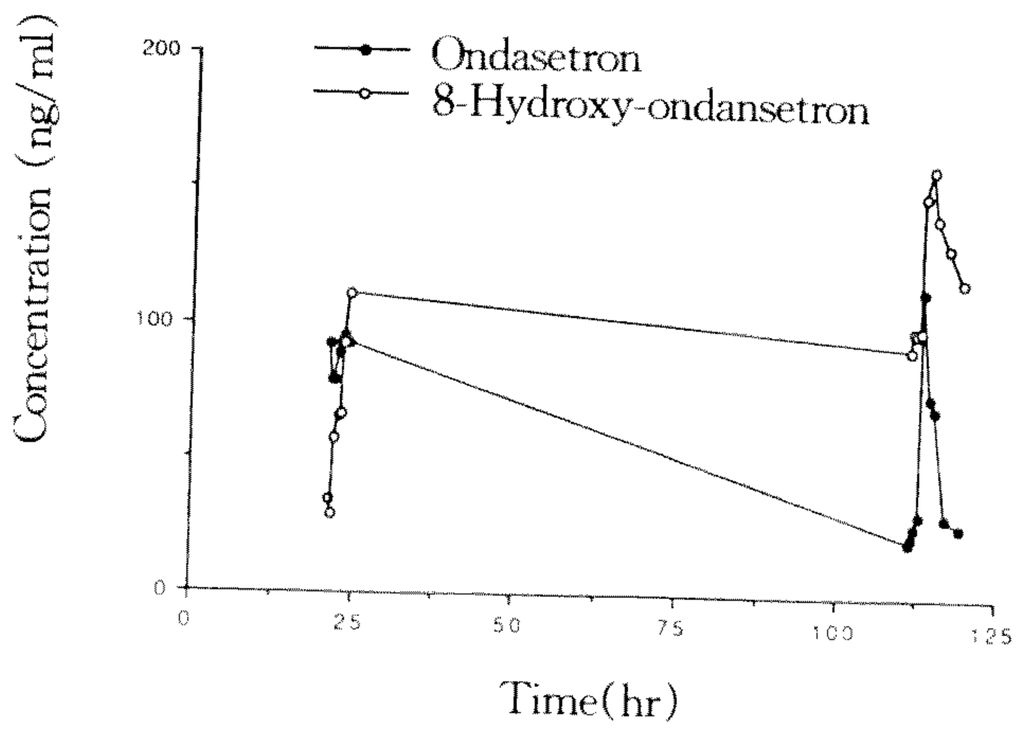

Figure 6. Ondansetron and 8-hydroxy-metabolite Plasma Levels in a Nasopharyneal Carcinoma Patient After Administered a 8 mg/day i.v. Infusion Loading Dose and Followed by Another t.i.d. 8 mg Tablet for 4 days

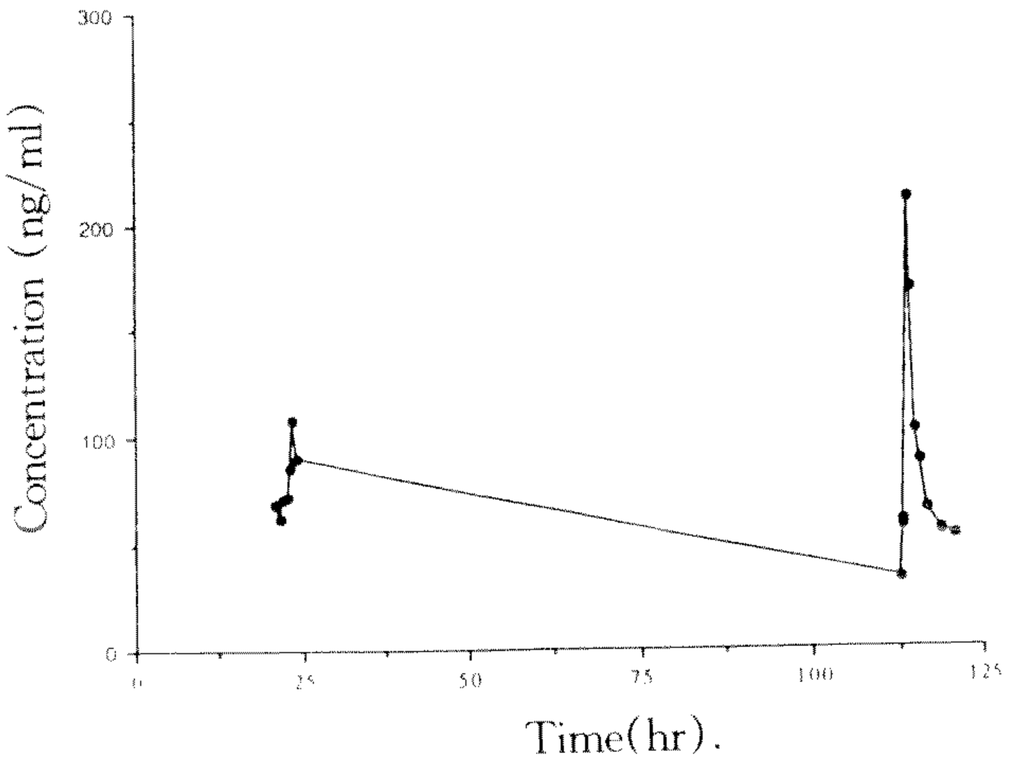

Figure 7. Ondansetron Plasma Levels in a Nasopharyneal Carcinoma Patient After Administered a 8 mg/day i.v. Infusion Loading Dose and Followed by Another t.i.d. 8 mg Tablet for 4 days, No Metabolite Was Detected in This Patient

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Ondansetron 及其代謝物之血漿濃度測定及其在多劑量藥動學之應用

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摘　要

Ondansetron 爲 5-HT_3接受器之拮抗劑，在臨床上可用來治療因癌症化學療法、放射性療法及手術後所引發的噁心、嘔吐。本文報告一種簡單、敏感、精確的高效液相層析法，用來分析血漿中 Ondansetron 及其代謝物（7-hydroxy-ondansetron 、8-hydroxy-ondansetron）的濃度，此分析方法是用 CN 管柱，以乙氰：0.0125 M pH4.7 醋酸氨緩衝液（2:8, V/V）之混合溶液爲移動相，並以 UV305nm 爲檢測波長並使用傳統的溶劑萃取法處理血液樣品。結果顯示 Ondansetron 於 20-800 ng/ml，二種代謝物於 100-800 ng/ml 的濃度範圍中，有很好的線性關係（$r > 0.999$）及精確度（同日內和異日間的變異係數皆於 10%左右），其最低可測得 Ondansetron 及 2 個代謝物濃度分別爲 5ng/ml、10 ng/ml 及 10ng/ml。本法已成功地應用至臨床癌症病人 Ondansetron 多劑量藥動學之研究。