The acidic layers that separated from the carbon tetrachloride layer were washed with carbon tetrachloride, neutralized with aqueous ammonia and re-extracted with methylene chloride. The methylene chloride extracts were combined, dried, and evaporated to dryness. The residue was crystallized from a mixture of isopropyl alcohol and ether to give 0.17 g (26.8% based on unrecovered **8**) of **2b**, mp 123—126°. The material was identical with an authentic sample.[12]

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# Analysis of 5-Chloro-7-iodo-8-quinolinol Conjugates by High Performance Liquid Chromatography

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An analytical method of 5-chloro-7-iodo-8-quinolinol (Clioquinol or Chinoform) (CF) conjugates was established by high performance liquid chromatography.

The chromatographic conditions were as follows: column, SAX (50 cm × 2 mm i.d.); mobile phase, I (0.02M borate buffer–0.1M KCl, pH 9.5), II (0.02M borate buffer–0.5M $NaClO_4$, pH 9.5); gradient, 0→25% $\left(\frac{\text{II}}{\text{I}+\text{II}}\right)$ at 3%/min; column pressure, 500 psi; flow rate, 1 ml/min; detector, ultraviolet-detector at 254 mμ. Urine samples of a man administered CF were injected directly to the column, and the major metabolite, CF glucuronide was determined, while very small amounts of CF sulfate was detected.

Recently, the metabolism of 5-chloro-7-iodo-8-quinolinol (Clioquinol or Chinoform) (CF) in a man and various animals has been studied by many investigators to clarify the cause of SMON (Subacute-Myelo-Optico-Neuropathy). In the course of this study, we developed the micro-analysis of CF and its conjugates in biological fluids by gas chromatography equipped with electron capture detector.[2]

It is a very sensitive and reliable method, but somewhat complicated. Then, the establishment of simple and convenient method was required in the analysis of biological samples which contain comparatively large amounts of CF conjugates such as urine.

It has been shown by Anders, *et al.*[3] that high performance liquid chromatography is very useful for the analysis of glucuronides and sulfates in the study of drug metabolism, because they could be analyzed directly without hydrolysis and derivatization.

In this work, we tried to use high performance liquid chromatography for the analysis of CF glucuronide (CF-G) and CF sulfate (CF-S) in human urine.

### Materials and Apparatus

CF-G and CF-S were synthesized according to Matsunaga's[4] and Chen's[5] methods.

**Apparatus**——DuPont 830 Liquid Chromatograph equipped with ultraviolet detector was used.

**Column**——"Zipax" SAX (DuPont) was packed in a stainless tube (50 cm × 2 mm i.d.). Other conditions are described in the figures.

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1) Location: *Hongo, Bunkyo-ku, Tokyo.*

2) Z. Tamura, M. Yoshioka, T. Imanari, J. Fukaya, J. Kusaka and K. Samejima, *Clin. Chim. Acta*, **47**, 13 (1973); C. Chen, K. Samejima and Z. Tamura, *Igaku no Ayumi* (Japan), **84**, 195 (1973).
3) M.W. Anders and J.P. Latorre, *J. Chromatog.*, **55**, 409 (1971).
4) I. Matsunaga and Z. Tamura, *Chem. Pharm. Bull.* (Tokyo), **19**, 1056 (1971).
5) C. Chen, K. Samejima and Z. Tamura, *Chem. Pharm. Bull.* (Tokyo), **21**, 911 (1973).

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## Results

Separation of CF-G and CF-S was examined on SAX column and operating conditions were conducted as shown in Fig. 1. Gradient elution was used for the simultaneous analysis of CF-G and CF-S, since the latter has much stronger affinity to the column, and $NaClO_4$ in secondary eluent remarkably shortened the retention time of CF-S to complete the analysis within 15 min. In this condition, the peak heights of CF-G and CF-S were proportional to the quantity of them in the range of 0.05—1.0 μg respectively, but we could not analyze CF, which was retained on the column.

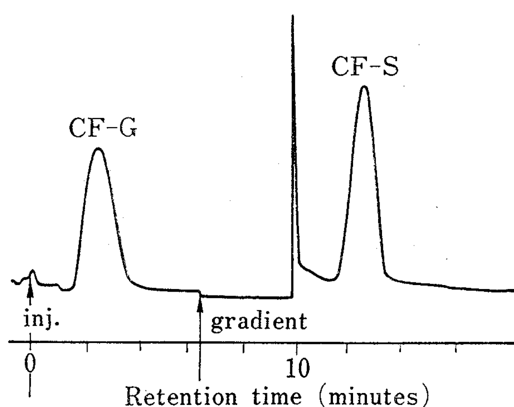


Fig. 1. Chromatogram of Chinoform Conjugates

operating conditions
apparatus: DuPont 830 Liquid Chromatograph
column: SAX (50 cm × 2 mm i.d.)
temperature: room temp.
mobile phase:
I: 0.02M borate buffer–0.1M KCl (pH 9.5)
II: 0.02M borate buffer–0.5M $NaClO_4$ (pH 9.5)
gradient $\left(\frac{I}{I+II}\right)$ 0→ 25%, at 3%/min
column pressure: 500 psi
flow rate: 1 ml/min
detector: UV photometer at 254 mμ

In order to apply this method to the study of CF conjugates in urine of a man administered CF, 4 μl of control urine was injected to the column directly and no peak interfered the analysis of CF conjugates as seen in Fig. 2a. Five hundred mg of CF was administered orally to four healthy subjects and their urine were analyzed by our method. As shown in the typical chromatogram (Fig. 2b), the most part of conjugates in urine was CF-G, while very small amounts of CF-S was detected. The cumulative excretion rate curves of CF-G (Fig. 3) demonstrated that about 10—20% of administered CF was excreted in urine as CF-G within 24 hr.

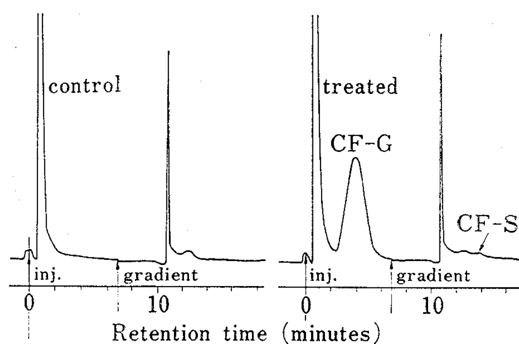


Fig. 2. Chromatogram of Chinoform Metabolites in Human Urine

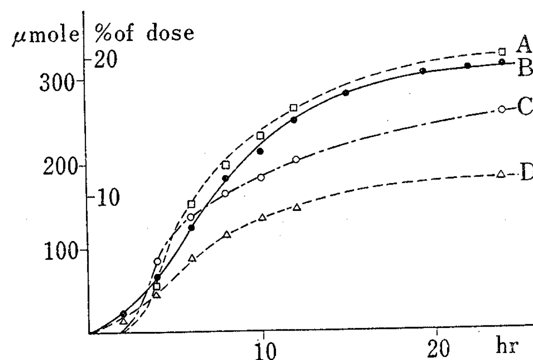


Fig. 3. Cumulative Urinary Excretion of Chinoform Glucuronide (CF-G)

subjects: A (sex: M, age: 25), B (M, 51), C (M, 44) D (M, 36)

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