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Note

Rapid monitoring of chloramphenicol in microsamples of plasma by capillary gas chromatography with electron-capture detection

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Chloramphenicol (CAP), a broad-spectrum antibiotic [1], has been reported to be effective in the treatment of typhoid and paratyphoid fevers and, particularly when it occurs in neonates and infants, for the treatment of meningitis caused by *Haemophilus influenzae* type b, which is resistant to ampicillin [2]. Especially in the case of infants, methods for the analysis of CAP should be able to cope with as little as 10 μl of sample, which can be obtained by fingertip puncture. High-performance liquid chromatographic (HPLC) methods [2–6] and gas chromatography (GC) with electron-capture detection (ECD) [7–9] are recommended for the measurement of CAP plasma levels. All these methods require a liquid–liquid extraction step.

This paper describes the isolation of CAP from tiny serum samples by solid-phase extraction (SPE) methods. A new silylation reagent, trimethylsilyl N,N-dimethylcarbamate (DMCTMS), is used to obtain a volatile diTMS-CAP derivative amenable to GC. Selective detection is achieved with ECD coupled to a fused-silica capillary column of cross-linked methyl silicone.

EXPERIMENTAL

Materials

Carbopack B HT 60/80 (Cat. No. 2-0274) graphitized carbon was obtained from Supelco (Gland, Switzerland), and 100 mg was tightly packed into a 1-ml polypropylene filtration tube (Cat. No. 5-7023) between two polyethylene

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discs with 20-μm pores. The carbon was washed with methylene chloride, acetone and methanol (50 ml of each). LC-18 tubes (1 ml) containing 100 mg of C_{18} sorbent were purchased from Supelco (Cat. No. 5-7002).

Chloramphenicol and *m*-chloramphenicol (m-CAP) were obtained from the Chemische Landesuntersuchungsanstalt (Offenburg, F.R.G.). The silylation reagent DMCTMS was prepared by Dr. D. Knausz (ELTE University, Budapest, Hungary) or purchased from Fluka (Buchs, Switzerland; Cat. No. 39 860).

All the organic solvents were of HPLC grade and used as purchased from Merck (Darmstadt, F.R.G.). The internal standard working solution was prepared by diluting 100 μl of stock solution of m-CAP (1.5 mg/ml in acetonitrile) in 100 ml of water. Pure CAP and m-CAP standards can be purchased from Parke-Davis (Freiburg, F.R.G.). Monochloro-CAP (Columbia Chemicals, Columbia, DC, U.S.A.) is also a suitable internal standard.

Sample preparation

C_{18} adsorption. CAP was isolated on the C_{18} reversed-phase packing as described in ref. 7. Freshly collected blood from donors was spiked with CAP at 5, 10, 25 and 50 μg/g, and then incubated at 37°C [6]. After 60 min the resulting mixture was centrifuged and the supernatant collected. Depending on the detection mode, 10 μl (ECD) or 100 μl (flame ionization detection) of this serum sample were applied to the LC-18 tube after it had been primed with 2 ml of methanol and 5 ml of water. An addition of 100 μl or 1.00 ml of the internal standard solution followed the sample application, then the tube was rinsed with 2 ml of water. The tube was vacuum-dried for 2 min, and the isolates were eluted into a 3.7-ml screw-capped vial by two 500-μl aliquots of acetone. The eluate was evaporated to dryness under a gentle stream of nitrogen at room temperature.

Silylation. This was performed with 100 μl of DMCTMS at 50°C for 15 min in the closed vial. Excess silylation reagent was hydrolysed by shaking the vial's content for 1 min with 2 ml of water and 100 μl of isooctane. After separation, the upper organic layer contained the diTMS derivatives of CAP and m-CAP.

Graphitized carbon black (GCB) adsorption. The Carbopack B HT tube was sequentially washed with 2 ml of 2-propanol, 2 ml of methanol and 5 ml of water. After sample application all steps were the same as described above, except that, before elution, the GCB packing was washed with 2 ml of *n*-hexane. The eluate (in this case, 1.50 ml of 2-propanol and 1.50 ml of methanol) was collected into a 3.7-ml vial, evaporated to dryness at 50°C and derivatized as above.

Chromatographic system

A Hewlett-Packard Model 5730A gas chromatograph was equipped with a split–splitless injection system, a dual flame ionization and electron-capture

detection system, two HP Model 3390A integrators and a remote start–stop module.

An HP ultra-performance fused-silica column (25 m × 0.2 mm I.D. with 0.11 μm cross-linked methyl silicone, Cat. No. 19 091A-002) was used throughout. The carrier gas was hydrogen at a head-pressure of 1 bar, and the split vent flow-rate was set to 50 ml/min. During split injection the injector temperature was 250°C, and the oven temperature was increased from 150 to 260°C at 10°C/min. All detectors were kept at a temperature of 300°C. The sample size was always 1 μl.

RESULTS AND DISCUSSION

Serum sample preparation

Prior to liquid–liquid extraction [1] or direct on-column (HPLC) injection [6], precipitation of plasma proteins is a common step to stop any further metabolic reaction and to obtain a clean supernatant, in order to prevent the build-up of solids at the column head. However, CAP is ca. 50% bound to plasma proteins, and so is likely to precipitate along with the denatured proteins. All these shortcomings are overcome by the use of solid-phase extraction. In good accord with the results of Kim et al. [5], Carbopack B HT, a graphitized carbon, was found to be of use when CAP was isolated from food samples [10]. Also, DMCTMS was used for the silylation of CAP. This reagent reacts only with hydroxyl groups in relatively mild conditions, it is very inexpensive and its by-products (carbon dioxide and dimethylamine), as well as the reagent itself, are volatile compounds that do not obscure any important peaks on the chromatogram. The diTMS-CAP is very stable even at low concentrations in isooctane saturated with water. When the extract is left to float on water, only 4% of the CAP is lost during a month at room temperature.

Although flame ionization detection is not sensitive enough for the determination of plasma CAP (at least 100 μl of sample are required, with splitless injection) it was clear from these chromatograms that the extra washing step with *n*-hexane in the GCB clean-up process produced an extract free of any fat-soluble compounds. This step is feasible only with graphite-like materials, which possess unique adsorption characteristics [5].

Chromatographic analysis

The aim of this study was to develop a rapid and sensitive method capable of working with only tiny amounts of blood samples. Flame ionization detection needs at least 100 μl of serum sample and a special (splitless) injection technique, which is not an option on every instrument. Also, this injection technique makes the chromatographic run longer than in the split mode, because the starting temperature must be lower to ensure the solvent effect and the final temperature must be higher to elute every artifact. ECD, however,

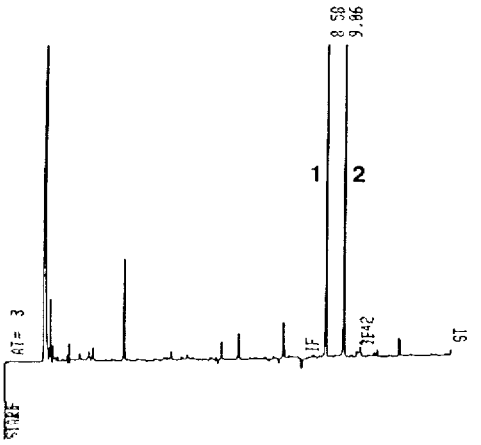

Fig. 1. ECD chromatogram of a serum sample (10 μl) spiked with 10 ppm CAP (split injection), after clean-up with Carbopack B HT. Peaks: 1 = m-CAP; 2 = CAP.

TABLE I

RECOVERIES OF KNOWN AMOUNTS OF CHLORAMPHENICOL FROM SPIKED BLOOD SAMPLES

GCB packing, 10 μl of serum sample; capillary GC-ECD; $n = 3$.

Amount spiked (μg/g)	Amount recovered (mean ± S.D.) (μg/g)	Recovery (%)
5	4.40 ± 0.2	88
10	9.00 ± 0.2	90
25	22.05 ± 0.2	88
50	43.05 ± 0.3	86

has high sensitivity toward the CAP, which allows the use of as little as 10 μl of sample and split injection (Fig. 1). The recovery values of known amounts of CAP added to blood samples are listed in Table I. A mean value of 88% was calculated over the concentration range 5–50 μg CAP per g blood. This is 3% less than the value published by Kim et al. [5], who pointed out the influence of the tube I.D. (more precisely, the ratio of the bed height versus I.D.) on the 'dynamic adsorptivity' (recovery). These results indicate that the most significant factor is the 'compactness' of the packing bed. The overall precision of the internal standard method is indicated by the relative standard deviation of 2.1%.

CONCLUSION

This study revealed the usefulness of GCB sorbents for trace enrichment of plasma CAP. The fused-silica capillary column and derivatization technique

with DMCTMS provide unique resolution and selectivity to separate CAP from any possible interfering drug administered simultaneously. The ECD made it possible to use only very small amounts of blood sample.

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