DETERMINATION OF PROCAINE, BENOXINATE AND DIBUCAINE IN BODY FLUIDS BY GAS CHROMATOGRAPHY-SURFACE IONIZATION DETECTION

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原　報

DETERMINATION OF PROCAINE, BENOXINATE AND DIBUCAINE IN BODY FLUIDS BY GAS CHROMATOGRAPHY-SURFACE IONIZATION DETECTION

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表面電離検出ガスクロマトグラフィーによる体液中プロカイン，ベノキシネートならびにジブカインの測定

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Summary

Procaine, benoxinate and dibucaine were found to be detected with high sensitivity by gas chromatography (GC)-surface ionization detection (SID). The three drugs showed excellent linearity in the range of 100-1000 pg in an injected volume. The detection limit of each drug was 100-200 pg (5-10 ng per ml of a sample). A detailed procedure for isolation of the local anaesthetics from human whole blood and cerebrospinal fluid (CSF) by the use of Sep-Pak C_{18} cartridges, before the GC-SID, is also presented. The recovery of the three drugs, which had been added to whole blood or CSF, was 70-100%.

Key words : Analytical toxicology ; Local anaesthetics ; Procaine ; Benoxinate ; Dibucaine ; Gas chromatography (capillary) ; Surface ionization detection ; Sep-Pak C_{18} cartridges

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Introduction

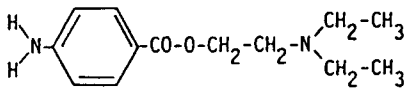
Surface ionization detection (SID) for gas chromatography (GC) was first introduced by Fujii and Arimoto in 1985 [1] . It was reported very specific and sensitive especially to compounds having tertiary amino groups in their structures, which form dissociative species at a low ionization potential. Application of GC-SID to analytical toxicology has recently been started and only a few studies on aprindine [2] and tricyclic antidepressants [3] have been reported. In our recent report [4] , we have presented that lidocaine, mepivacaine and bupivacaine give extremely high sensitivity by GC-SID ; their detection limit was as low as 5-10 pg in an injected volume. In this paper, we report that procaine, benoxinate and dibucaine also show satisfactory sensitivity by GC-SID.

Experimental

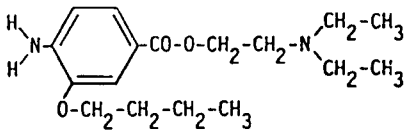
Materials

Chemical structures of the local anaesthetics used in this study are shown in Fig. 1. Pure powder of procaine-HCl and dibucaine-HCl was obtained from Sigma (St. Louis, MO, USA), and that of benoxinate-HCl from Santen Pharmaceutical (Osaka, Japan). Sep-Pak C_{18} cartridges were purchased from Waters (Milford, MA, USA). Other common chemicals used were of the highest purity commercially available. Whole blood was obtained from healthy subjects and cerebrospinal fluid (CSF) from cadavers at forensic autopsy.

Procaine



Benoxinate



Dibucaine

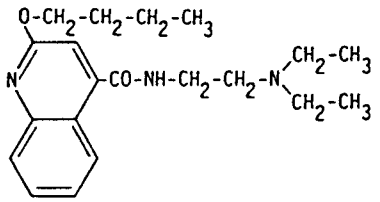


Fig. 1. Chemical structures of three local anaesthetics used in this study.

Isolation with Sep-Pak C_{18} cartridges

The drugs were extracted with Sep-Pak C_{18} cartridges according to our previous report [5] . For pretreatment of a cartridge, 10ml of methanol and 10ml of distilled water were passed through it.

A lml volume of whole blood or CSF, with or without addition of drugs (50 ng each), was mixed with 7ml distilled water and then with 3ml of 1M sodium bicarbonate. The mixture was loaded on the pretreated Sep-Pak cartridge at a flow rate not greater than 5ml/min. It was washed with 10ml of water twice, and finally 3ml of chloroform/ethanol (4 : 1) was passed through it to elute the local anaesthetics, which were collected in a vial. The eluate consisted of a major amount of an organic layer (lower phase) and a minor amount of an aqueous layer (upper phase) ; the latter was discarded by aspiration with a Pasteur pipette. The organic layer was evaporated to dryness under a stream of nitrogen, and the residue dissolved in 100μl of methanol. A 2μl aliquot was subjected to GC analysis.

GC conditions

A Shimadzu GC-15A instrument, equipped with a SID system with an intermediately polar fused silica DB-17 capillary column (30m × 0.32 mm i. d., film thickness 0.25μm, J & W Scientific, folsom, CA, USA) and a split-splitless injector, was used. The GC conditions were : column temperature, 120-280°C (2min hold at 120°C and 8°C/min) ; injection temperature, 200°C ; helium flow-rate, 22 cm/s. The SID conditions were : heating current through the platinum emitter, 2.2A ; emitter temperature, ca. 600°C ; ring electrode bias voltage, +200V with respect to the collector electrode. The samples were injected in the splitless mode at 120°C of the column temperature and splitter was opened after 2min.

Results

Figure 2 shows gas chromatograms by SID for 50ng each of procaine, benoxinate and dibucaine, which had been added to 1ml of whole blood and CSF and extracted with Sep-Pak C_{18} cartridges. The three drugs were completely separated from biological impurities on gas chromatograms. The recovery of procaine, which had been added to whole blood was about 70%, but that of benoxinate and dibucaine was nearly 100%. For CSF samples, the recovery of every drug was close to 100%. The baselines remained steady during the increase in column temperature.

Figure 3 shows calibration curves for the three drugs. They showed satisfactory linearity in the range of 100-1000 pg in an injected volume. The equation and r values for the curves were : $y = 0.00581x + 0.0578$, $r = 0.9992$ for procaine ; $y = 0.00541x - 0.0434$, $r = 0.9997$ for benox-

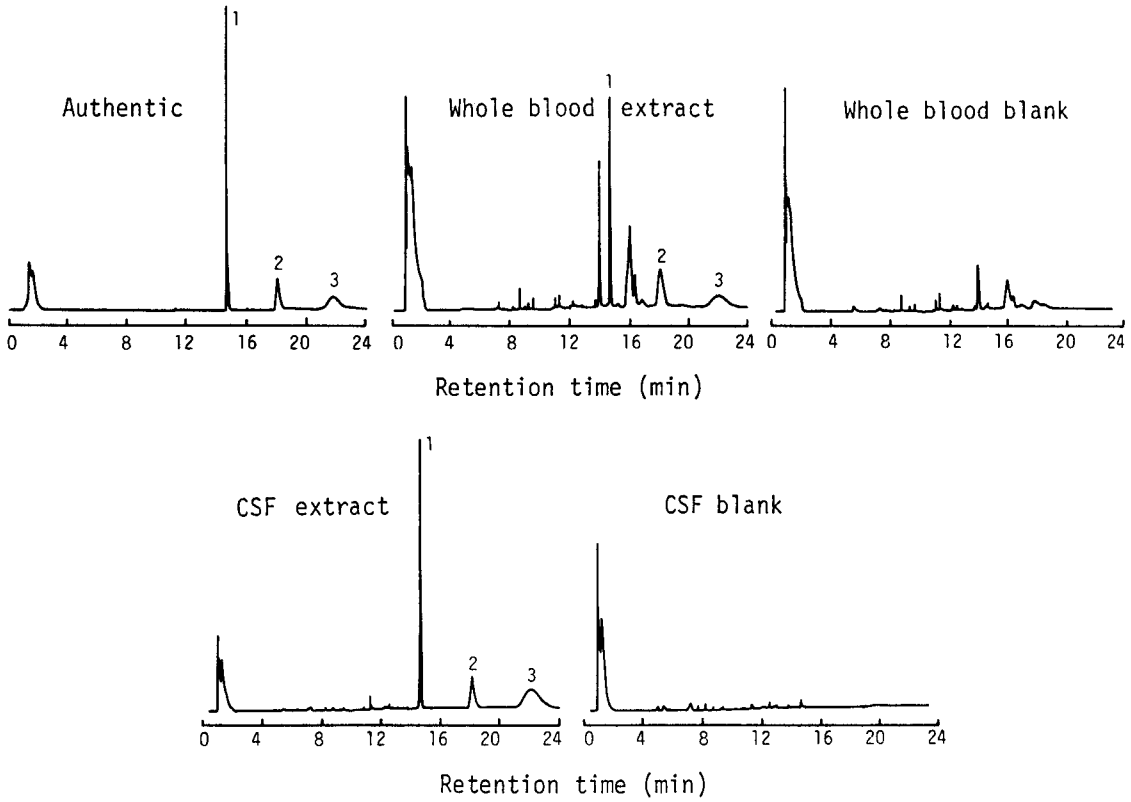


Fig. 2. Capillary GC-SID for procaine (peak 1), benoxinate (peak 2) and dibucaine (peak 3) extracted from whole blood and CSF, and for each background with use of Sep-Pak C_{18} cartridges. GC was carried out with a DB-17 fused silica capillary column (30m × 0.32 mm i. d., film thickness 0.25μm). Its conditions were : column temperature, 120-280°C (8°C/min) ; injection temperature, 200°C ; helium flow-rate, 22 cm/s. The samples were injected in the splitless mode at 120°C of column temperature, and splitter was opened after 2 min. The mixture of the three local anaesthetics (50ng each) was added to 1ml whole blood or CSF.

inate ; and $y = 0.00811x - 0.6080$, $r = 0.9967$ for dibucaine. The detection limit was 5-10ng per ml of a sample (100-200 pg in an injected volume).

Discussion

In this study, we have been able to detect procaine, benoxinate and dibucaine with high sensitivity by GC-SID (Figs. 2 and 3), though the response of this detector to the above drugs is one order of magnitude lower than that to lidocaine, mepivacaine and bupivacaine [4] . We carefully compared the sensitivity of the GC-SID with that of nitrogen-phosphorus detection (NPD) for the present procaine, benoxinate and dibucaine ; the sensitivity of GC-SID was

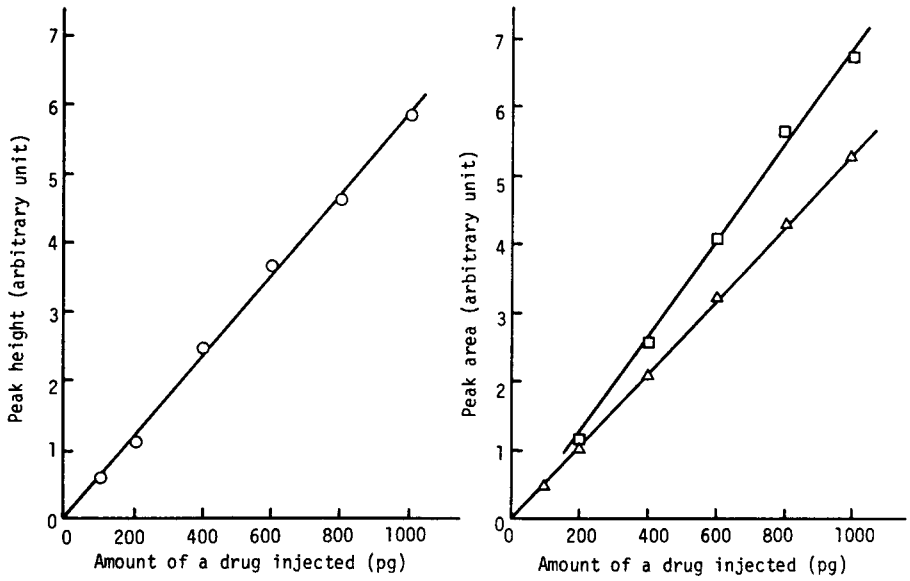


Fig. 3. Calibration curves by GC-SID for procaine (○), benoxinate (△) and dibucaine (□). GC conditions were as specified in Fig. 2.

10-100 times higher than that of GC-NPD when judged with their signal-noise ratios (unpublished observation). The baselines for GC-SID remained steady as the column temperature was increased (Fig. 2), but those for GC-NPD were elevated remarkably (unpublished observation).

As materials for analysis, we have used whole blood and CSF in the present study, and have got relatively clean backgrounds for them (Fig. 2). Plasma and serum also gave clean backgrounds by GC-SID [2, 3], but urine caused many impurity peaks in backgrounds [3], which were probably due to excretion of many methylated metabolites of amines into urine.

We have isolated the local anaesthetics with Sep-Pak C_{18} cartridges and eluted them with chloroform/ethanol (4 : 1). In our previous study on other local anaesthetics, we used chloroform/methanol (9 : 1) [4]. The merits of the minor change in solvent composition are that the recovery of the present three local anaesthetics is improved from 70% to 100%, and thus the results are less variable, and that the backgrounds are cleaner with chloroform/ethanol (4 : 1) than with chloroform/methanol (9 : 1) (unpublished observation).

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