DETERMINATION OF DEXTROMETHORPHAN AND DIMEMORPHAN IN BODY FLUIDS BY GAS CHROMATOGRAPHY WITH SURFACE IONIZATION DETECTION

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原　　報

DETERMINATION OF DEXTROMETHORPHAN AND DIMEMORPHAN IN BODY FLUIDS BY GAS CHROMATOGRAPHY WITH SURFACE IONIZATION DETECTION

Hiroshi SENO[a]*, Hideki HATTORI[b], Takumi IIZUMI[a], Takeshi KUMAZAWA[a] and Osamu SUZUKI[a]

[a] *Department of Legal Medicine, Hamamatsu University School of Medicine, 3600 Handa-cho, Hamamatsu 431-31, Japan*
[b] *Department of Legal Medicine, Aichi Medical University, Nagakute-cho, Aichi 480-11, Japan*

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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デキストロメトルファンとジメモルファンの測定

妹尾　洋[a]，服部秀樹[b]，飯泉　工[a]，熊沢武志[a]，鈴木　修[a]

[a]浜松医科大学法医学教室　〒431-31 静岡県浜松市半田町3600番地
[b]愛知医科大学法医学教室　〒480-11 愛知県愛知郡長久手町大字岩作字雁又21番地

Summary

Dextromethorphan and dimemorphan were found detectable with high sensitivity by gas chromatography with surface ionization detection. Dextromethorphan was measured against dimemorphan as internal standard, and dimemorphan measured against dextromethorphan conversely. Both drugs showed linearity in the range of 50—400 pg in an injected volume. The detection limit was *ca.* 20 pg on column (1 ng per ml of a sample). A detailed procedure for isolation of the drugs from human whole blood and urine with use of Sep-Pak C_{18} cartridges is also presented. The recovery of the drugs was almost 100 %.

Key words : Dextromethorphan ; Dimemorphan ; Gas chromatography ; Surface ionization detection ; Sep-Pak C_{18} cartridges

* Correspondence should be addressed to Hiroshi Seno.

Introduction

Surface ionization detection (SID) for gas chromatography (GC) was first introduced by Fujii and Arimoto in 1985 [1]. It was suggested to be very sensitive and specific to tertiary amines. Recently, reports have appeared on GC-SID for some drug groups [2-6]. In this paper, we have found that dextromethorphan and dimemorphan, antitussives of morphine analogues, can also be detected by GC-SID with high sensitivity.

Experimental

Materials

Chemical structures of dextromethorphan and dimemorphan are shown in Fig. 1. Dextromethorphan hydrobromide was purchased from Sigma Chemical Co., St. Louis, MO, USA, and dimemorphan phosphate was kindly donated from Yamanouchi Pharmaceutical Ind. Co., Ltd., Tokyo. Sep-Pak C_{18} cartridges were purchased from Waters Associates, Milford, MA, USA. Other common chemicals were of the highest purity commercially available. Whole blood and urine were obtained from healthy subjects.

Isolation with Sep-Pak C_{18} cartridges

Sep-Pak C_{18} cartridges were pretreated by passing 10 ml of chloroform/ethanol (9 : 1), 10 ml of acetonitrile, and 10 ml of distilled water, and this procedure was repeated more than 5 times.

To 1 ml of whole blood or urine, with and without addition of drugs (20 ng of each), 10 ml of distilled water and 1 ml of 1 M $NaHCO_3$ were added. The sample solution was then loaded on a pretreated Sep-Pak cartridge at a flow rate not greater than 5 ml/min. It was washed

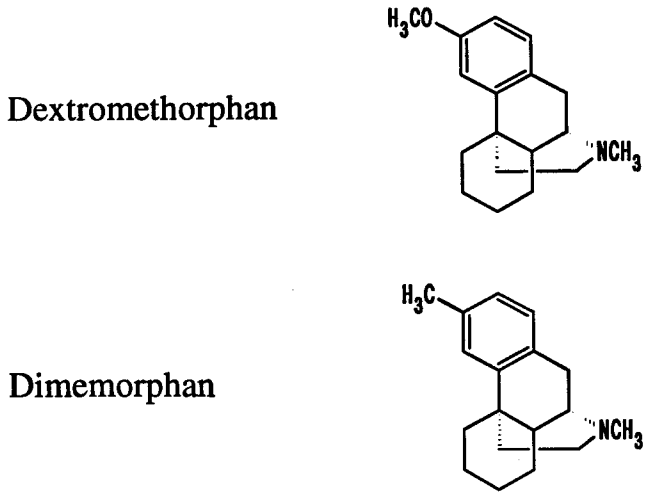

Fig. 1. Chemical structures of dextromethorphan and dimemorphan.

with 20 ml of distilled water, and finally 3 ml of chloroform/ethanol (9 : 1) was passed through it to elute the drugs. The eluate was collected in a vial, and the minor aqueous layer (upper phase) was discarded by aspiration with a Pasteur pipette. The organic layer was evaporated to dryness under a stream of nitrogen. The residue was dissolved in 100 μl of methanol and a 2-μl aliquot of it was subjected to GC analyses.

GC conditions

GC was carried out on a Shimadzu GC-14A gas chromatograph with an SID system and on a Hewlett-Packard Model 5890 gas chromatograph with nitrogen-phosphorus detection (NPD). A DB-17 fused silica capillary column (30 m×0.32 mm i.d., film thickness 0.25 μm, J & W Scientific, Folsom, CA, USA) and a split-splitless injector were used for both GC instruments. The GC conditions for both instruments were : column temperature, 100—280 ℃ (2 min hold at 100 ℃ and 10 ℃/min) ; injection and detector temperature, 280 ℃ ; helium flow rate, 3 ml/min. The SID conditions were : heating current through the platinum emitter, 2.2 A ; emitter temperature, *ca.* 600 ℃ ; ring electrode bias voltage, +200 V with respect to the collector electrode. The samples were injected in the splitless mode at 100 ℃ of the column temperature and the splitter was opened after 2 min.

Results and discussion

Figure 2 shows gas chromatograms by GC-SID for 20 ng each of dextromethorphan and dimemorphan, which had been added to 1 ml of whole blood and urine, and extracted with Sep-Pak C_{18} cartridges. Small interfering peaks overlapped the drug peaks, but their contamination gave almost no problem. The recovery was close to 100 %.

Figure 3 shows calibration curves for the drugs. Dextromethorphan was measured against dimemorphan (400 pg on column) as internal standard (IS), and dimemorphan measured against dextromethorphan (400 pg on column), conversely. They showed linearity in the range of 50—400 pg in an injected volume. The equation and r values for the curves were : $y=0.00198x+0.0164$, $r=0.9992$ for dextromethorphan ; $y=0.00321x+0.0368$, $r=0.9998$ for dimemorphan. The detection limit was 1 ng per ml of a sample (20 pg in an injected volume) for both drugs.

We compared the sensitivity of the GC-SID with that of GC-NPD for the present drugs. The sensitivity of GC-SID was approximately 5 times higher than that of GC-NPD when judged with signal-to-noise ratios. In comparison with sensitivity of GC-flame ionization detection with a packed column in the literature [7], in which dextromethorphan was extracted from cough-cold syrups, the sensitivity of the present GC-SID is more than two orders of magnitude higher.

We have extracted the drugs from whole blood and urine samples using Sep-Pak C_{18} cartridges.

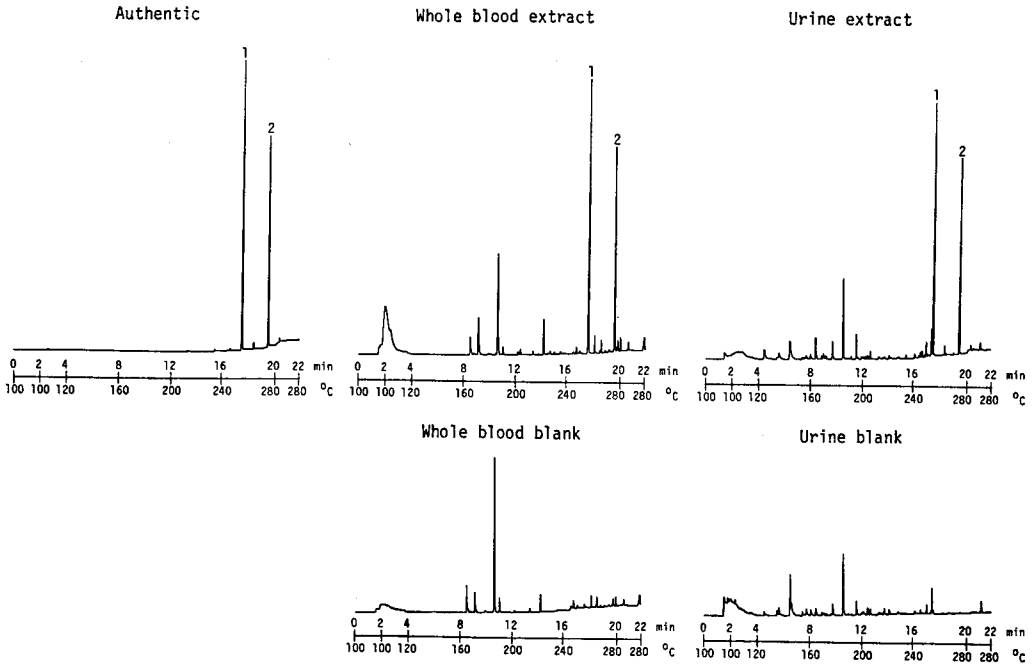

Fig. 2. Capillary GC-SID for dimemorphan (peak 1) and dextromethorphan (peak 2) extracted from whole blood and urine, and for each background with use of Sep-Pak C_{18} cartridges. The mixture of 20 ng of each drug was added to 1 ml of samples. GC was carried out with a DB-17 fused silica capillary column (30 m×0.32 mm i.d., film thickness 0.25 μm). Its conditions were : column temperature 100—280 ℃ (10 ℃/min) ; injection and detector temperature, 280 ℃ ; helium flow rate, 3 ml/min.

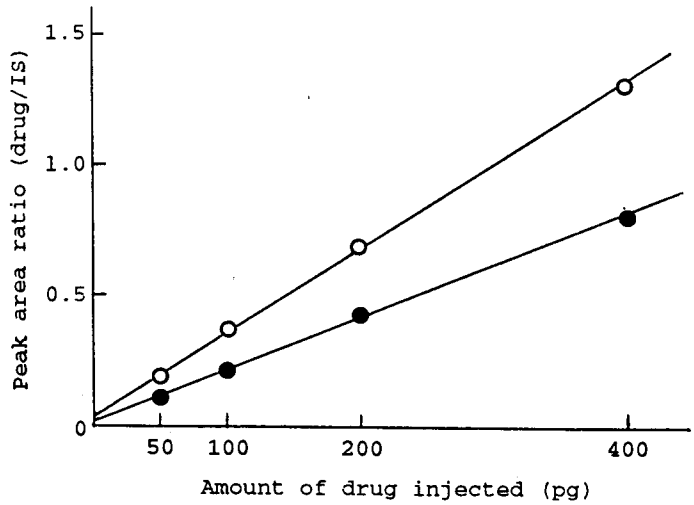

Fig. 3. Calibration curves by GC-SID for dimemorphan (○) and dextromethorphan (●). The vertical axis shows the peak area ratio (drug to IS). GC conditions were as specified in Fig. 2. Dimemorphan was quantitated against dextromethorphan as IS and *vice versa*.

To our knowledge, no reports are available on the use of Sep-Pak C_{18} cartridges to isolate dextromethorphan and dimemorphan. Our Sep-Pak method is simpler and more rapid as compared with the liquid-liquid extraction usually being employed before GC analyses. We have used a mixture of chloroform and ethanol as the elution solvent, though methanol or acetonitrile is recommended according to the manufacturer's manual. The merits to use chloroform are that recoveries are much better, backgrounds much cleaner, and evaporation time of the eluate much shorter [8].

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