DETERMINATION OF TRIHEXYPHENIDYL IN BODY FLUIDS BY GAS CHROMATOGRAPHY WITH SURFACE IONIZATION DETECTION

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原　　報

DETERMINATION OF TRIHEXYPHENIDYL IN BODY FLUIDS BY GAS CHROMATOGRAPHY WITH 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トリヘキシフェニジルの定量

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Summary

Trihexyphenidyl (benzhexol, Artane®), a well known antiparkinsonism drug, could be detected with high sensitivity by gas chromatography with surface ionization detection (GC-SID) using diphenylpyraline as internal standard. The calibration curve showed excellent linearity in the range of 15 to 500 pg on column. The detection limit was about 10 pg on column (0.5 ng/ml). Solid phase cartridges of Sep-Pak C_{18} were useful for extraction of the drugs from body fluids because of good recovery and purification.

Key words: Trihexyphenidyl; Diphenylpyraline; Antiparkinsonism drug; Gas chromatography; Surface ionization detection; Sep-Pak C_{18} cartridges

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Introduction

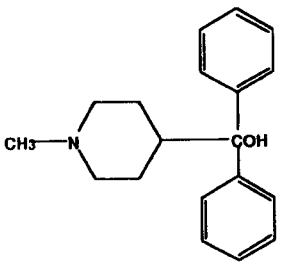
Trihexyphenidyl (benzhexol) is widely used for Parkinson's disease and drug-induced parkinsonism [1]. In the use of the drug, side effects, such as cycloplegia, constipation and urinary retention, sometimes appear as results of its anticholinergic action, especially for aged patients. In some patients, its doses of more than 12 mg daily may produce severe mental disturbance and excitement [2]; thus its monitoring during administration is important. Rubinstein emphasized the abuse potential of anticholinergic drugs because of their euphoric effects [3]; actually some trihexyphenidyl abusers were reported [3–5].

Several reports of its determination by gas chromatography (GC) with flame ionization detection (FID) [6, 7], flame thermionic detection (FTD) [8] and mass spectrometry [9, 10] were presented. In 1985, Fujii and Arimoto first introduced surface ionization detection (SID) for GC [11, 12]; it was shown to be very suitable for the sensitive and specific detection of tertiary amines [13]. In this report, we briefly demonstrate that trihexyphenidyl can be determined by GC-SID with high sensitivity and specificity.

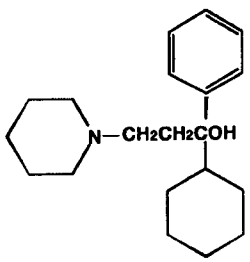
Experimental

Materials

Chemical structures of trihexyphenidyl and diphenylpyraline (internal standard) are shown in Fig. 1. Both drugs were purchased from Sigma Chemical Co. (St Louis, MO, USA). Sep-Pak C_{18} cartridges were from Waters Associates (Milford, MA, USA) and a DB-1 fused silica capillary column (15m x 0.32 mm i.d., film thickness 0.25 μm) from J & W Scientific (Folsom, CA, USA). Other chemicals used were of analytical grade. Whole blood and urine were obtained from healthy subjects.



Trihexyphenidyl



Diphenylpyraline

Fig. 1. Chemical structures of trihexyphenidyl and diphenylpyraline (IS).

Extraction with Sep-Pak C_{18} cartridges

Procedures for pretreatment of Sep-Pak C_{18} cartridges and for extraction of trihexyphenidyl and diphenylpyraline spiked in whole blood and urine samples were the same as those of our previous report [14]. At the initial step of the extraction, one ml sample was used for each analysis; finally the extract

residue was dissolved in 100 μl methanol and a 2-μl aliquot was injected into the GC port.

GC conditions

GC was carried out on a Shimadzu GC-14 B gas chromatograph equipped with SID (Shimadzu Co., Kyoto). The GC conditions were: column temperature, 150−300 ℃ (1 min hold at 150 ℃); injection temperature, 230 ℃; and helium flow rate, 3 ml/min. The samples were injected in the splitless mode at a column temperature of 150 ℃ and the splitter was opened after 1 min. The SID conditions were: detector temperature, 280 ℃; heating current through the platinum emitter, 2.2 A; emitter temperature, about 600 ℃; and ring electrode bias voltage, + 200 V with respect to the collector electrode.

Results

Figure 2 shows profiles of chromatograms by GC-SID for urine or whole blood samples, spiked and not spiked with trihexyphenidyl and diphenylpyraline (12.5 ng each/ml samples). The retention time were 8.4 min for trihexyphenidyl and 7.6 min for diphenylpyraline (IS). Any impurity peak did not overlapped the drug peaks. The recovery of trihexyphenidyl from urine and whole blood was 102 % and 82.2 %, respectively; that of diphenylpyraline 105 % and 90.6 %, respectively.

Figure 3 shows a calibration curve for trihexyphenidyl against diphenylpyraline as IS (250 pg on column). It showed good linearity in the range of 15−500 pg on column. The equation and the regression coefficient were $y=0.0674+0.00374\ x$ and $r=0.996$. The detection limit was about 10 pg on column (0.5 ng/ml sample).

Discussion

This is the first report for detecting trihexyphenidyl by GC-SID and for extracting the drug with Sep-Pak C_{18} cartridges.

Many years ago, Lovejoy [6] and Bargo [7] reported GC analysis of trihexyphenidyl with packed columns and FID; their detection limits were about 200 ng and 400 pg on column, respectively. More recently, Owen et al. [10] have reported a selective capillary GC method with FTD (nitrogen-phosphorus detection); their detection limit was insisted to be 5 pg on column. The present GC-SID showed comparable sensitivity (about 10 pg of detection limit) to that of Owen et al. [10]. The higher selectivity of SID toward tertiary amino groups gives backgrounds cleaner and more stable than those for GC-FTD [13].

The daily dose of trihexyphenidyl hydrochloride is 1 to 15 mg, and the drug level declines rapidly after oral administration [2]. It is possible to detect trihexyphenidyl 24 h after 10 mg

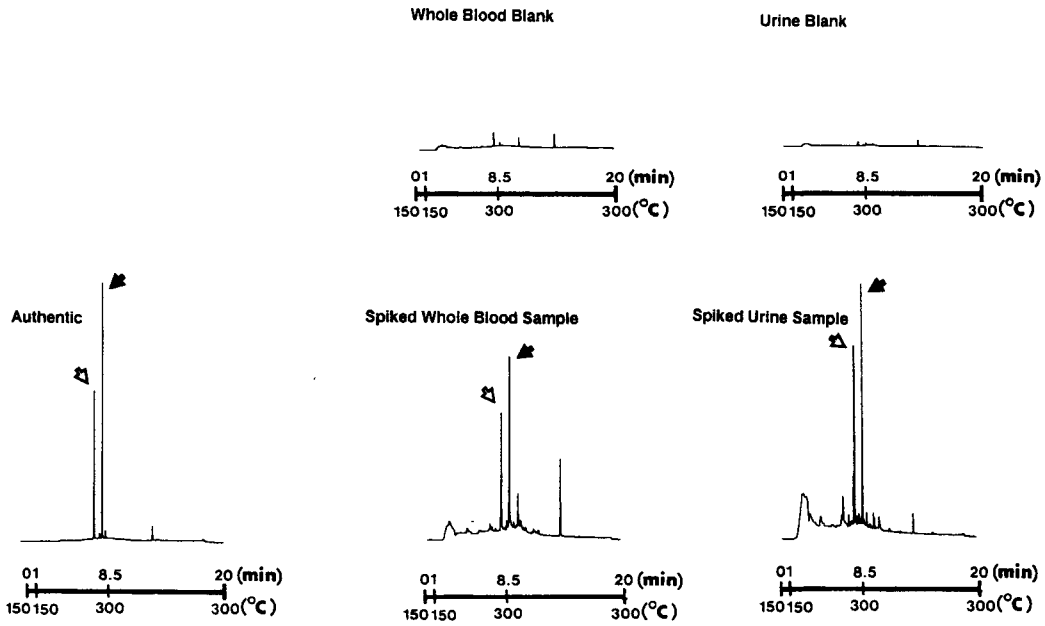


Fig. 2. Capillary GC-SID for trihexyphenidyl (filled arrow) and diphenylpyraline (open arrow) extracted from whole blood and urine, and their backgrounds, using Sep-Pak C_{18} cartridges. GC was carried out with a fused silica DB-1 capillary column (30 m x 0.32 mm i.d., film thickness 0.25 mm). GC conditions were: column temperature, 150—300 ℃ (20 ℃/min); injection temperature 230 ℃; detector temperature, 280 ℃; helium flow rate, 3 ml/min. The samples were injected in the splitless mode at a column temperature of 150 ℃ and the splitter was opened after 1 min. The mixture of trihexyphenidyl and diphenylpyraline (12.5 ng each) was added to 1 ml of whole blood or urine.

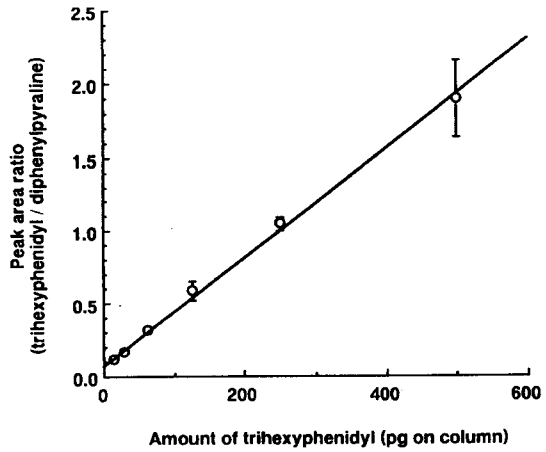


Fig. 3. Calibration curve for trihexyphenidyl with diphenylpyraline as IS. Open circles and bars show the peak area ratios of trihexyphenidyl to IS (250 pg on column) and SD, respectively. GC conditions were as specified in Fig. 2.

oral administration, since the concentration at this time was about 2 ng/ml [9]. Our GC-SID method has enabled measurement of trihexyphenidyl in samples of lower drug levels and smaller sizes extending its possibility in forensic and clinical toxicology.

Acknowledgment

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