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原　　報

SENSITIVE QUANTITATION OF CLOZAPINE IN BODY FLUIDS BY GAS CHROMATOGRAPHY WITH SURFACE IONIZATION DETECTION*

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表面電離ガスクロマトグラフィーによる体液中クロザピンの高感度定量

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Summary

Clozapine, an atypical neuroleptic, has been found measurable with high sensitivity by gas chromatography with surface ionization detection (GC-SID) using perlapine as internal standard. The calibration curve for clozapine showed excellent linearity in the range of 1.25 to 40 ng / ml whole blood (25 to 800 pg on-column), and the detection limt was about 1 ng / ml body fluid (20 pg on-column). Solid-phase extraction with Sep-Pak C_{18} was useful for rapid purification of drugs from body fluids; the recoveries were above 88 % for whole blood or urine samples.

Key words: Clozapine; Perlapine; Gas Chromatography (GC); Capillary GC; Surface ionization detection; Sep-Pak C_{18} cartridges

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Introduction

Clozapine is a tricyclic dibenzodiazepine derivative and classified as an "atypical" neuroleptic drug. Its pharmacological action is primarily *via* interaction with dopamine receptor in the limbic system [1]. Clozapine was reported to have higher affinity for dopamine D_4 receptor rather than for D_2 receptor [2]. It has also high affinity for other multiple catecholamine and indolamine receptors [3]. Although clozapine does not induce extrapyramidal side-effects [4] and seems to ameliorate negative symptoms of schizophrenia very effectively [3,5], it was not permitted for its clinical use until February 1990 in the United States, because of agranulocytosis, a rare but severe side-effect [6,7]. Although it has not been permitted to be prescribed in Japan yet, there have been several reports of clozapine intoxication over the world [8–10]. In this paper, we have developed a simple and sensitive method for quantitation of clozapine from body fluids by gas chromatography with surface ionization detection (GC-SID) using perlapine as internal standard (IS).

Experimental

Materials

Clozapine was purchased from Research Biochemicals International (Natick, MA, USA) and perlapine from Takeda Chem. Ind. Ltd. (Osaka). Chemical structures of the drugs are shown in Fig.1. Other chemicals used were of analytical grade. Sep-Pak C_{18} cartridges were purchased from Waters Associates (Milford, MA, USA), and a DB-1 fused-silica capillary column (30 m × 0.32 mm i.d., film thickness 0.25 μm) from J&W Scientific (Folsom, CA, USA). Whole blood and urine were obtained from healthy subjects.

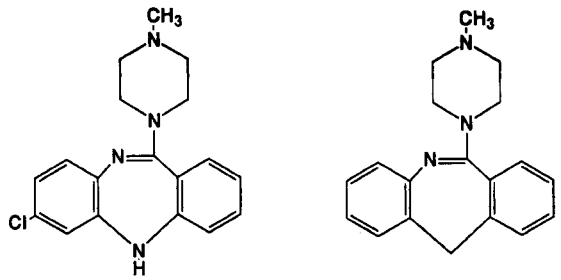

Fig. 1. Chemical structures of clozapine and perlapine (IS).

Extraction Sep-Pak C_{18} cartridges

Sep-Pak C_{18} cartridges were pretreated by passing 10 ml of chloroform, 10 ml of methanol and 10 ml of distilled water, and this procedure was repeated 5 times.

To 1 ml of whole blood or urine, with or without addition of drugs (5 or 20 ng of clozapine and 10 ng of perlapine), 8 ml of distilled water and 1 ml of 1 M $NaHCO_3$ were added. The sample solution was then loaded on the pretreated Sep-Pak C_{18} cartridges, which were washed with 20

ml of distilled water. The drugs were eluted with 3 ml of chloroform/methanol (9:1). The organic layer of the eluent 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 analysis.

GC conditions

GC-analyses were carried out on a Shimadzu GC-14 B gas chromatograph equipped with an SID system (Shimadzu Corp., Kyoto). The GC conditions were: column temperature, 150−300 ℃ (1 min hold at 150 ℃ and 20 ℃ / min); injection temperature, 240 ℃; and helium flow rate, 3 ml/min. The SID conditions were: detector temperature, 280 ℃; heating current through platinum emitter, 2.2 A; emitter temperature, about 600 ℃; and the ring electrode bias voltage, + 200 V with respect to the collector electrode. The samples were injected in the splitless mode at a column temperature of 150 ℃ and the splitter was opened after 1 min.

Results and discussion

Figure 2 shows gas chromatograms by GC-SID for 20 ng of clozapine and 10 ng of perlapine (IS), which had been added to 1 ml of whole blood or urine, and extracted with Sep-Pak C_{18} cartridges. The retention times were 12.2 min for clozapine and 9.7 min for perlapine (IS). There were no impurity peaks which interfered with peaks of clozapine and perlapine.

Table 1 shows percent recoveries and their coefficients of variation (CV) of clozapine and perlapine (IS) from 1 ml of whole blood or urine samples. The recoveries were above 88 % from urine and whole blood.

A calibration curve was drawn for clozapine, which had been added to and extracted from 1 ml of whole blood with 10 ng of perlapine as IS. The curve was linear in the range of 1.25 to 40 ng/ml blood (25 to 800 pg on column if recovery is 100 %). The equation and coefficient of regression (r) were: $y=-0.0492 + 0.0617x$ and $r=0.998$. The detection limit (signal-to-noise ratio = 3) was about 1 ng/ml body fluid (20 pg on column).

This is the first report showing that clozapine is detectable by GC-SID. In the previous reports, clozapine was measured by thin layer chromatography (TLC) [11], GC-nitrogen phosphorus detector (NPD) [12,13], high-performance liquid chromatography with ultraviolet detection (HPLC-UV) [14−18], and HPLC-amperometric detection [19]. The detection limits in these reports were 5 ng /ml plasma by TLC [11], 1−2 ng/ml by GC-NPD [13], 2−5 ng/ml by HPLC-UV [14,15,17,18] and 2 ng/ml by HPLC-amperometric detection [19]. The sensitivity in this present report was higher or comparable to those by other methods.

Several toxic effects of clozapine, such as unusual eosinophilic myocarditis and myocarditis like changes were reported [8−10]. Because the anticholinergic effect of clozapine [5] may

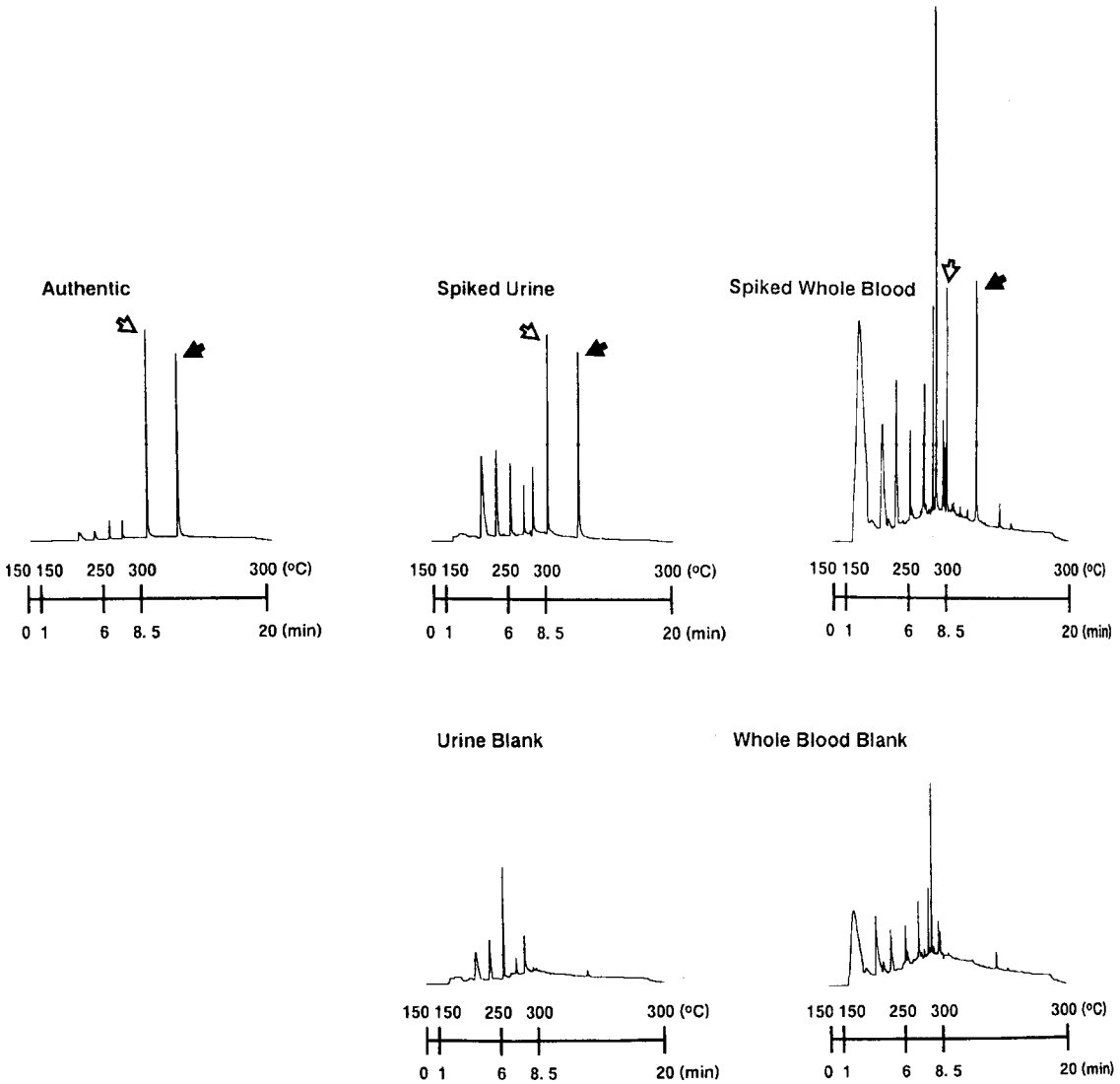

Fig. 2. Chromatograms of capillary GC-SID for clozapine (filled arrows) and perlapine (IS, open arrows) extracted from whole blood and urine samples. The amounts of clozapine and perlapine spiked to each 1 ml sample were 20 ng and 10 ng, respectively.

Table 1. Persent recoveries of clozapine and perlapine (IS) from body fluids extracted with Sep-Pak C_{18} cartridges

Compound	Percent recovery			
	Whole blood		Urine	
	mean	CV	mean	CV
Clozapine (20 ng/ml)	88.1	10[a]	100	6.9[a]
Clozapine (4 ng/ml)	97.8	17[a]	93.2	14[a]
Perlapine (10 ng/ml)	91.9	18[b]	91.2	9.9[b]

[a] n = 6 ; [b] n = 12

enhance its cardiac toxicity, it seems very important to monitor its serum level regularly during its administration. Our present method would be useful in both fields of forensic toxicology and clinical toxicology.

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