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原　　報

RAPID ISOLATION WITH SEP-PAK C_{18} CARTRIDGES AND CAPILLARY GAS CHROMATOGRAPHY OF KETOTIFEN AND MEQUITAZINE IN BODY FLUIDS

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ケトチフェン，メキタジンの Sep-Pak C_{18} カートリッジによる迅速分離法とキャピラリーガスクロマトグラフィー

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Summary

A simple and rapid method for isolation of ketotifen and mequitazine with Sep-Pak C_{18} cartridges from human whole blood and urine is presented. The detection of the drugs was made by a non-polar capillary gas chromatography with a nitrogen-phosphorus detector. The drug containing samples, after mixing with slightly alkaline buffer solution, were directly applied to the cartridges and eluted with chloroform/methanol (9 : 1). The recoveries of ketotifen were close to 100 % for both whole blood and urine samples, and those of mequitazine were 50.0 % and 70.7 %, respectively. Both drugs could be separated from impurities on the gas chromatograms. The detection limit of each drug was about 400 pg in an injected volume.

Key words : Ketotifen ; Mequitazine ; Capillary gas chromatography ; Nitrogen-phosphorus detector ; Sep-Pak C_{18} cartridges

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Introduction

Ketotifen and mequitazine are classified into anti-asthmatic and anti-histaminic drugs, respectively, and both widely used for softening catarrhal symptoms of cold and allergic rhinitis ; they are occasionally encountered in forensic science practice. In this paper, we present a simple and rapid isolation procedure for ketotifen and mequitazine from biological impurities using Sep-Pak C_{18} cartridges and their detection by capillary gas chromatography (GC) with a nitrogen-phosphorus detector (NPD).

Experimental

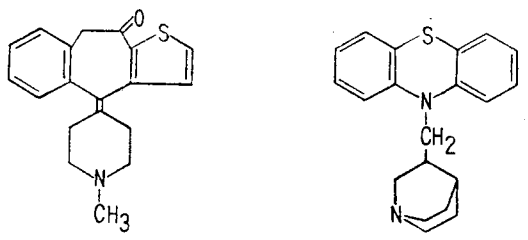
Materials

Ketotifen fumarate salt was purchased from Sigma (St. Louis, MO, USA), and mequitazine was kindly donated by Toyo Jozo (Tokyo, Japan). Their chemical structures are shown in Fig. 1. 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 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 chloroform/methanol (9 : 1) mixture, 10 ml acetonitrile and 10 ml distilled water ; this procedure was repeated three times.

A whole blood or urine sample (1 /m), containing 100 ng each of ketotifen and mequitazine, was mixed with 10 ml distilled water, and 2 ml 0.8M $NaHCO_3$. The mixture was poured into a pretreated Sep-Pak C_{18} cartridge at a speed not greater than 5 ml/min. The cartridge was washed with 20—30 ml distilled water. Finally, 3 ml chloroform/methanol (9 : 1) was passed through it to elute the drugs, and was 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



Ketotifen Mequitazine

Fig. 1. Chemical structures of ketotifen and mequitazine.

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 methanol, and a 2-μl aliquot of it was subjected to GC analysis.

GC conditions

GC was carried out on a Hewlett Packard 5890 Series II gas chromatograph with a NPD, a DB-1 capillary column (15 m × 0.32 mm i.d., film thickness 0.25 μm, J & W Scientific, Folsom, CA, USA), and a split-splitless injector. The GC conditions were : column temperature, 150—240 ℃ (5 ℃/min) ; injection and detector temperature, 260 ℃ ; helium flow rate, 3 ml/min. The samples were injected in the splitless mode at a column temperature of 150 ℃, and splitter was opened after 1 min.

Results

Figure 2 shows gas chromatograms for human whole blood and urine extracts with and without addition of 100 ng each of ketotifen and mequitazine to 1 ml samples. The retention times for ketotifen and mequitazine were 13.6 and 16.0 min, respectively. Both drugs were separated from impurity peaks for both blood and urine. The backgrounds were much cleaner for urine than for whole blood. To remove the background noises, the Sep-Pak cartridge, after loading of samples, was washed with 20—30 ml methanol/water (1 : 10) solution ; it was effective to reduce the big impurity peaks around 3 min of retention time, but caused lower recovery of mequitazine.

The recoveries for ketotifen were close to 100 % for both whole blood and urine samples. That of mequitazine was 70.7 % for urine samples, but for whole blood samples, it was 50.0 %, which was probably due to firm binding of the drug to blood proteins.

The detection limit was about 400 pg for both drugs in an injected volume (20 ng per ml of whole blood or urine).

Discussion

To our knowledge, this is the first trial to isolate ketotifen and mequitazine in biological samples with Sep-Pak C_{18} cartridges. In the previous reports, they were extracted with organic solvents by repeating shakings and centrifugations [1-4]. These extraction methods were much more complicated and time-consuming than the prsent Sep-Pak method.

Various concentration of methanol or acetonitrile are being used for elution of various compounds from Sep-Pak C_{18} cartridges according to the manufacturer's manual. In the present experiments, we used chloroform/methanol (9 : 1) as an elution solvent. One of the merits of the use of

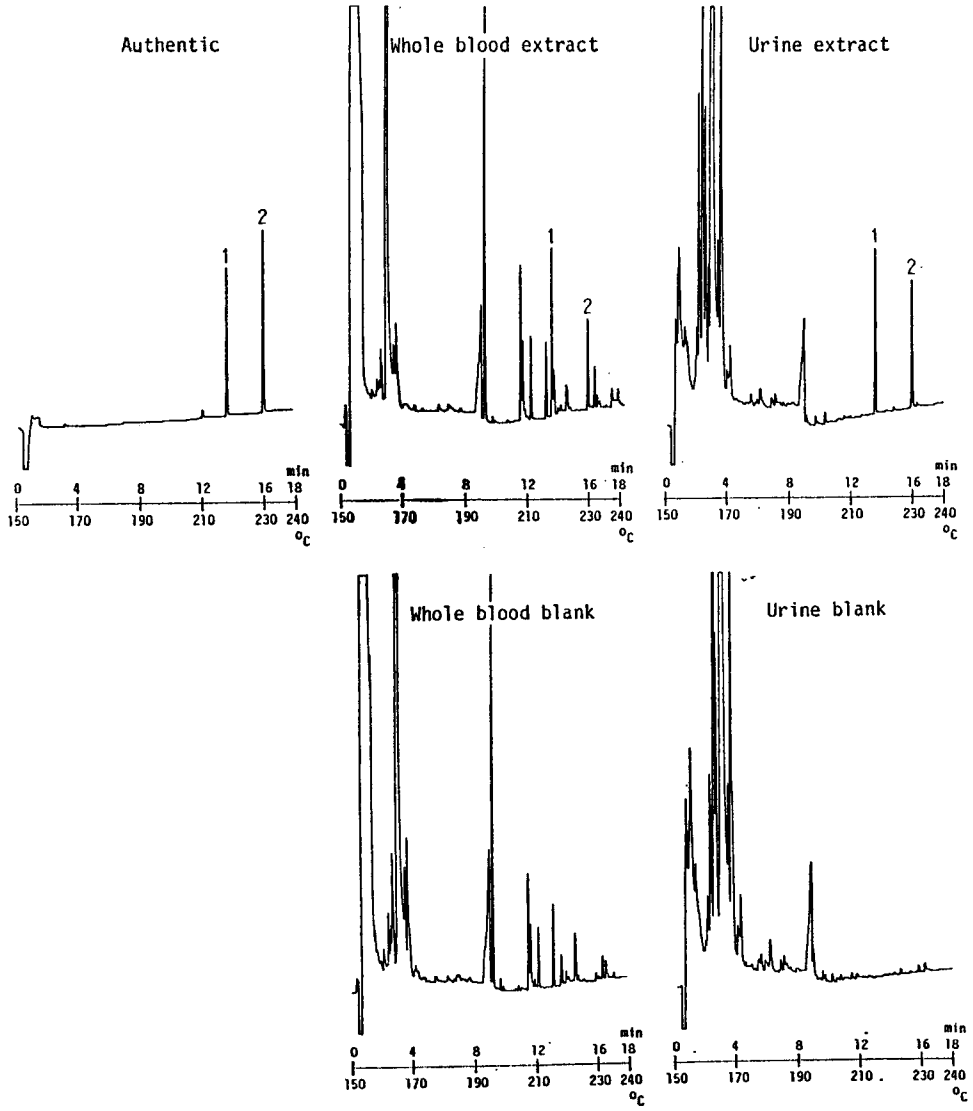


Fig. 2. Capillary GC with a NPD for ketotifen (peak 1) and mequitazine (peak 2) extracted from whole blood and urine, and for each background with use of Sep-Pak C_{18} cartridges. GC was carried out with a DB-1 fused silica capillary column (15 m × 0.32 mm i.d., film thickness 0.25 μm). For its conditions, see text. The mixture of ketotifen and mequitazine (100 ng each) was added to 1 ml whole blood or urine.

chloroform/methanol (9 : 1) is that the time required for evaproration of the eluate is relatively short. The evaporation of the 3 ml chloroform/methanol (9 : 1) eluate required about 30 min, while that of 100 % methanol or acetonitrile eluates requires about 120 min [5]. Furthermore, the use of the present elution solvent gives cleaner backgrounds and much better recoveries than with methanol or acetonitrile.

Therapeutic doses of ketotifen and mequitazine are as low as 2—4 mg and 10 mg daily, respectively. Therapeutic plasma concentration of ketotifen was reported to be 0.6 ng/ml 2h after oral administration of 2 mg drug [6]. Toxic plasma levels of the drug were 16—122 ng/ml ; in a fatal case involving ketotifen, a postmortem blood concentration was 1.2 μg/ml [6]. The corresponding data for mequitazine, however, are not available in literatures to our knowledge. Therefore, it seems impossible to detect the low therapeutic levels of both ketotifen and mequitazine by the present method with a NPD, because it gave detection limit of about 400 pg (20 ng/ml body fluid) for both drugs. Our method should be used for measurements of toxic levels of these drugs in forensic and clinical toxicology. In an attempt to get higher sensitivity, we tried, for both drugs, GC with a new system surface ionization detector [7-9], which is said to be 10 times more sensitive than that with a NPD, but failed to get higher sensitivity for the drugs (unpublished observation).

In spite of the failure to get sufficient sensitivity for therapeutic levels of ketotifen and mequitazine, the present simple isolation procedure is recommendable as pretreatment for any analytical method of ketotifen and mequitazine measurements.

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