ANALYSIS OF METHYLBENACTYZIUM BROMIDE IN HUMAN URINE BY THIN-LAYER CHROMATOGRAPHY AND PYROLYSIS GAS CHR OMATO GRAPHY

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Summary

A rapid and simple method of utilizing thin-layer chromatography (TLC) and pyrolysis gas chromatography (PyGC) for the identification and determination of methylbenactyzium bromide in human urine was studied in this report. Methylbenactyzium bromide was extracted from urine with ODS-cartridge (Sep-Pak C_{18}), then spotted onto a silica gel 60 F_{254} TLC plate. After development, the separated spot of methylbenactyzium bromide was scraped and wrapped with a ferromagnetic foil without extraction by any organic solvents. The sample was applied into PyGC analysis. The optimum temperature for pyrolysis was 590°C. The main degradation product of methylbenactyzium bromide was identified as diphenylmethane in this procedure by gas chromatography/mass spectrometry (GC/MS). A calibration graph prepared by absolute calibration method showed a good linearity over the concentration range of 1-75 ìg/spot for methylbenactyzium bromide. The coefficient of variation obtained for eleven replicate analyses of the 3ìg /spot of standard methylbenactyzium bromide was 3.8%. The detection limit of this compound by this procedure was 0.1ìg /spot.

Key words: Methylbenactyzium bromide; Thin-layer chromatography; Pyrolysis gas chromatography; ODS-cartridge

Introduction

Methylbenactyzium bromide (2-diethylaminoethyldiphenylglycolate methylbromide, $C_{21}H_{28}BrNO_3$; 422.37) has been used as a spasmolytic for the treatment of gastrointestinal ulcer. The structure of methylbenactyzium is shown inFig.1.

As with other quaternary ammonium salts, it is very soluble in water and can not be extracted with any organic solvents. For the reason mentioned above, the separation of this drug from several biological fluids is quite difficult. Furthermore, the drug can not be extracted from scraped silica gel in preparative thin-

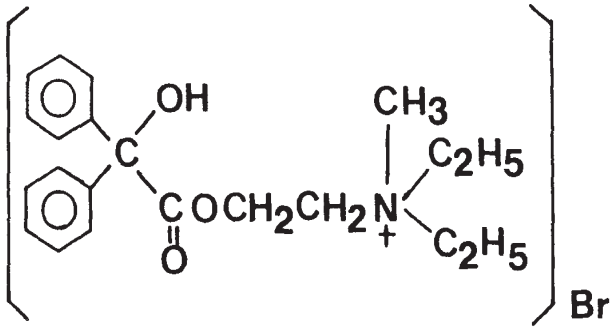

Fig 1 Chemical structure of methylbenactyzium bromide

layer chromatograph and gas chromatographic analysis is difficult because of its low volatility.

There are few reports concerning the analysis of methylbenactyzium. Ohtsubo et al. suggested in their report that this drug and other quaternary ammonium compounds were extracted by Bond-Elute CBA extraction cartridge and were analyzed with reversed phase ion pair chromatography [1].

For the analysis of other quaternary ammonium compounds, Ellin et al. reported high performance liquid chromatography (HPLC) of pyridostigmine in serum after purification with Sep-Pak C_{18} cartridge [2]. Charles et al. also reported the ion pair extraction of propantheline by perchloric acid and the analysis of it by HPLC [3]. Stevens et al. reported that suxamethonium in urine was extracted with ion pair extraction using bromothymol blue (BTB) and gas chromatography (GC) could be performed using ester exchange of this compound [4]. Castagnoli et al. also investigated the extraction of pancuronium in serum with potassium iodide, and direct inlet/chemical ionization mass spectrometry (DI/CI-MS)[5]. However, these methods were difficult to be conducted in forensic laboratories because of their handling difficulty.

Tsuchihashi et al. have reported the simultaneous extraction of the eight kinds of quaternary ammonium salt by ODS-cartridge; they also reported the analysis of these compounds with thin-layer chromatography (TLC). They also identified the pyrolyzed products by Curie point pyrolysis gas chromatography/mass spectrometry (PyGC/MS) [6]. Simple and easy extraction method was carried out, but large amounts of urine were required for analysis of these drugs and the interference of urine components were observed on its pyrogram [6]. In this report, the effluents from ODS-cartridge were purified by preparative thin-layer chromatography (PTLC), and the separated spot was scraped, and this fraction containing silica gel, was wrapped with ferromagnetic foil and was directly applied to PyGC analysis. This method was simple and the purification effect of PTLC would be expected to improve the detection limit of this drug, and considered to be useful in forensic science field.

Materials and Methods

Materials

Methylbenactyzium bromide was obtained from Yamanouchi Pharmaceutical Co. ,

Ltd. (Tokyo, Japan). Standard solution of this compound were prepared to add to drug-free human urine (pH 7.2), giving a concentration of 0.01-100 mg/ml. Other reagents used in this experiment were analytical grade.

Apparatus

Pyrolyzer. This was a JHP-3 type model Curie point pyrolyzer (Nippon Bunseki Kougyo, Tokyo, Japan), and the gas chromatograph a Shimadzu GC-7AG equipped with an CBP-10 fused silica capillary column with a film thickness of 0.25 ìm and dimensions of 50 m x 0.2 mm i.d. The GC conditions were: column temperature, 150-270°C (10°C/min); carrier gas, N_2; linear velocity, 30 cm/sec; split ratio, 1:60. Obtained data was processed with Shimadzu C-R3A data processor. TLC plate was Silica Gel 60 F_{254} (Merck, Darmstadt, FRG) and the ODS-cartridge was a Sep-Pak C_{18} obtained from Waters Assoc. (Milford, MA, U.S.A.).

Analytical procedures

One milliliter of urine sample was adjusted to pH 7.5 by saturated trisodium-phosphate solution, and was added to the ODS-cartridge pre-activated by introducing 5 ml of water, 5 ml of methanol and 20 ml of water into the cartridge, and the drug was retained in the cartridge. The cartridge was washed with 3 ml of water then eluted with 3 ml of methanol. The methanol fraction was dried in vacuum and 1 ml of methanol was added to the residue. An aliquot (10 ìl) of this solution was spotted onto the silica gel plate and developed with 5% formic acid/methanol/tetrahydrofuran (6:6:7, by vol.). The observed spot (RF value was 0.55) under UV irradiation (254 nm) was scraped and the scraped fraction corresponding to methylbenactyzium was wrapped with ferromagnetic foil (590 °C, 9 x 23 mm) and pyrolyzed for 3 s in a Curie point pyrolyzer.

Results and Discussion

Degradation products of methylbenactyzium by pyrolysis

One milliliter of 1 mg/ml of standard solution of methylbenactyzium bromide was added to pre-activated ODS-cartridge, the effluent was dried on ferromagnetic foil, and the residue on the foil was applied to a Curie point pyrolyzer. Two peaks of pyrolyzed products of methylbenactyzium were observed on its pyrogram under this condition. These products were assigned to benzophenone and diphenylmethane by gas chromatography/mass spectrometry (GC/MS). GC/MS spectra showed that the major product of purification followed by pyrolysis was diphenylmethane, and the second product being benzophenone. Contrary to this result, we have already reported that the major product of pyrolysis of this drug without PTLC purification was benzophenone [6J. The pyrograms obtained from these experiments are shown in Fig. 2.

The amount of diphenylmethane, the major degradation product of this procedure, was compared with that of the benzophenone, major pyrolyzed product produced without PTLC purification [6]. The detection limit determined by major pyrolyzed products after PTLC purification was twice higher than that without PTLC treatment.

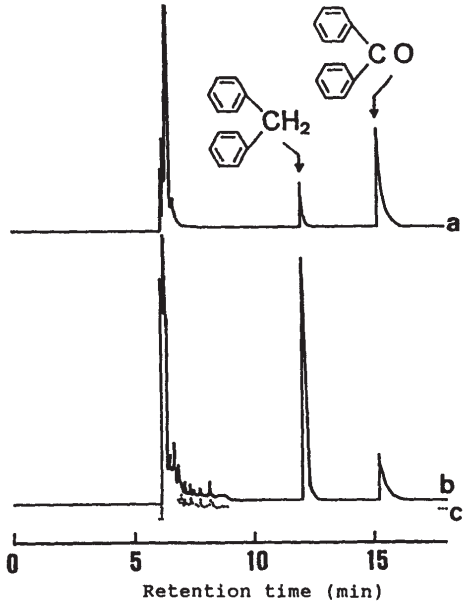

Fig 2 Pyrograms of methylbenactyzium bromide (a) urine sample effluent directly pyrolyzed (b) urine sample effluent pyrolyzed after PTLC (c) urine control effluent pyrolyzed after PTLC

One milligram of benzophenone or benzilic acid was spotted onto TLC plates and each fraction was scraped and pyrolyzed by the method mentioned above; diphenylmethane was not produced from benzophenone. On the other hand, diphenylmethane and benzophenone were produced from benzilic acid as in the case of pyrolysis of methylbenactyzium. From these results, diphenylmethane and benzophenone were presumed to be produced from benzilic acid as an intermediate.

Optimum pyrolysis period and temperature

After PTLC of 10 /ìg of methylbenactyzium, the fraction of this drug with silica gel was wrapped at 386, 445, 500, 590 and 650°C ferromagnetic foil and pyrolyzed. The amount of major product, diphenylmethane was measured by its peak area on the pyrogram. The results are shown in Fig. 3.

The higher the temperature of pyrolysis, the more abundant was the amount of major degradation product observed; however, the background from scraped silica gel also became higher. Overall, the most suitable pyrolysis temperature was 590°C.

Also the pyrolysis period was observed for 2, 3 and 4 s. The peak areas of the two degradation products on its pyrogram did not increase even with longer period of pyrolysis. Therefore, the pyrolysis of methylbenactyzium was conducted for 3s.

Effect of amounts of silica gel on the detection limits

Methylbenactyzium bromide (10 ìg) was spotted on TLC plates, and was scraped

from the plate containing this area with 1-10 mg (2-10 mm diameter) of silica gel, and PyGC was conducted. The results are shown in Fig. 4. Peak areas of degradation products were not effected by the amount of silica gel.

Calibration curve

According to this procedure, the calibration curve of methylbenactyzium was prepared using the peak ea of diphenylmethane; the linearity was observed within the range of 1-75 / ìg/spot. The calibration curve is shown in Fig. 5. The detection limit of methyibenactyzium by this method was 0.3 ìg, and was twice as high as that of direct pyrolysis of effluent from the cartridge without PTLC purification. Though methylbenactyzium was also detected by UV absorption (254 nm), and several color reagents, such as Dragendorff reagent and iodo-platinate reagent on TLC plate, the detection of limits of this drug by these procedures were lower than present method. When 11 repeated analyses of 3 /ìg /spot of standard solution of methylbenactyzium were conducted, the calibration coefficients were 3.8%.

Another quaternary ammonium salt drug

The urinary solution of other quaternary ammonium salts were applied to ODS-cartridge and the effluents were (1) directly applied to PyGC and (2) after PTLC purification, also analyzed by Pyr/GC, and the detection limits by both methods were investigated by comparing with the peak area of major pyrolyzed products on their mass fragmentogram. The peak area ratio of major pyrolyzed product of each compound ($S_{\text{method 2}}$ /$S_{\text{method 1}}$) of both methods were calculated [6]. These values were in distigmine (150%), pancuronium (807%), propantheline (341%), suxamethonium (65%), neostigmine (76%) and benzethonium (25%).

These results are summarized in Table 1.

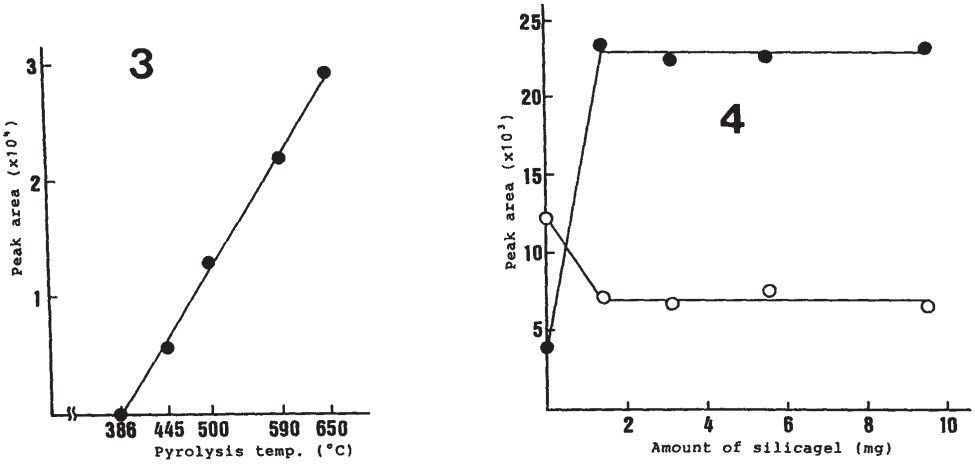

Fig. 3. Effect of pyrolysis temperature on peak area of major degradation product, diphenylmethane.

Fig. 4. Effect of amount of silica gel on peak area of degradation products; ●, diphenylmethane; ○, benzophenone.

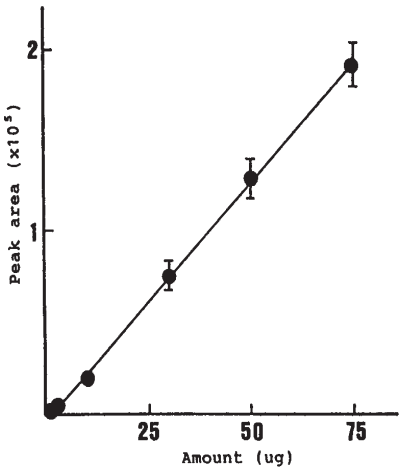

Fig 5 Calibration graph for methylbenactyzium bromide

Conclusions

The analytical procedure for one of the quaternary ammonium drugs, methylbenactyzium bromide, utilizing ODS-cartridge extraction, PTLC, and PyGC was investigated. In this method, the major degradation product of pyrolysis was assigned to diphenylmethane. The calibration curve was prepared using this peak area on its pyrogram. The optimum temperature and period of pyrolysis was 590°C, 3 s, respectively. Under these conditions, the amounts of degradation products were abundant, and the interference by background was not observed. The detection limit of methylbenactyzium under this condition was 0.3 ìg and the calibration graph showed linearity between 1 and 75 ìg. Furthermore, by this method, 6 other quaternary ammonium salts could be detected with good sensitivity.

TABLE 1

COMPARISON OF PyGC AND PTLC-PyGC FOR 7 QUATERNARY AMMONIUM COMPOUNDS

Compound	*PTLC-PyGC/PyGC (%)*
Distigmine bromide	150
Bethanechol chloride	n d
Pancuronium bromide	807
Propantheline bromide	341
Suxamethonium chloride	65
Neostigmine methylsulphate	76
Benzethonium chloride	25

n d , not detected

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