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Determination of pyridostigmine and the main degradation product in tablets by high-performance liquid chromatography

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Summary

A high-performance liquid chromatographic (HPLC) method for quantification of pyridostigmine bromide and its major degradation product, 3-hydroxy-1-methylpyridinium bromide, in tablets has been developed. The method involves the use of a polymeric column with acetonitrile-water (7.8:92.2 v/v) mixture and an ion-pair reagent as mobile phase. With the injection of 4.7 μg pyridostigmine bromide the limit of quantitation of the major degradation product is 0.01 μg which corresponds to 0.21% degradation. Blister packs of tablets from a single batch number, which had been stored under different conditions in Norway and Saudi Arabia, were analysed. Degradation was determined to be 1.4–1.6%. The age of the batch was about 3 years. The method is well suited for accelerated kinetic studies and routine control of aged pyridostigmine tablets.

Introduction

Quantitative determination of pyridostigmine from blood and urine samples has been carried out with various methods. The need for such procedures is based on the use of pyridostigmine in the treatment of myasthenia gravis and in anesthesia to curtail the muscular relaxation produced by non-depolarising neuromuscular blockade (Reynolds, 1982).

One of the previous methods described is based on a colorimetric determination (Nowell et al., 1962). Other methods are radioimmunoassay (Miller and Verma, 1989), gas-liquid chromatography (Chan et al., 1976), gas chromatography (Pohlmann et al., 1977; Davison et al., 1980) and thin-layer chromatography in combination with mass spectrometry (Stanley et al., 1988). The literature describes several HPLC methods for determination of pyridostigmine in biological fluids (Yakatan and Tien, 1979; De Ruyter et al., 1980; Ellin et al., 1982; Shih et al., 1986; Matsunaga et al., 1987; Ryabik and Ho, 1987; Yturralde et al., 1987; Michaelis, 1990). Two of these have been used to separate both pyridostigmine and its main metabolite 3-hydroxy-1-methylpyridinium bromide (THMP) (De Ruyter et al., 1980; Ellin et al., 1982). This metabolite corresponds to the major degradation product of pyridostigmine (Patel et al., 1980) (Fig. 1). Previous work (Patel

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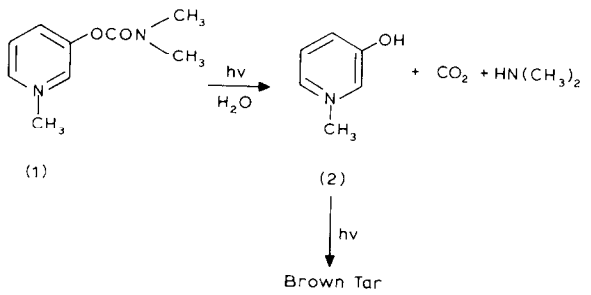

Fig. 1. Degradation of pyridostigmine (Patel et al., 1980).

et al., 1980) has shown that degradation occurs when a solution of pyridostigmine bromide (1) is subjected to simulated sunlight irradiation and/or boiling. The degradation products are THMP (2) and the gases carbon dioxide and dimethylamine. Further irradiation of THMP gives the solution a brown color. This is probably due to a developed brown tar, which has not been investigated successfully.

A HPLC method for determination of pyridostigmine and THMP in tablets based on repeated extractions has recently been published (Phi et al., 1991). The aim of our study was to develop an adequate method for the control of the tablets in stock combined with easy and rapid sample preparation.

In military medicine, pyridostigmine is a prophylactic against nerve gas poisoning. In case of mobilization soldiers in the Norwegian armed forces are equipped with tablets which contain pyridostigmine for prophylactic use over 1 week.

The Norwegian armed forces store the drugs in several small depots all over the country. To monitor stability and to predict the shelf life of pyridostigmine tablets, our laboratory is dependent on a reliable method for routine control of long-term storage of the tablets.

Materials and Methods

Sample preparation

A sample of 15 tablets from a blister pack of 21 tablets were crushed in a mortar. Three subsamples were withdrawn from each blister pack. A subsample consisted of the accurate weight of a 2.5 tablet (0.442 g), which was transferred to a 50.00 ml volumetric flask and diluted with the mobile phase. The solution was filtered through a 0.45 μm MFS® filter. A volume of 1.00 ml was transferred to a 20.00 ml volumetric flask and diluted with the mobile phase. The sample was then injected three times into the chromatograph.

Standard curves

Pyridostigmine A five-point standard curve with 1.66, 3.00, 5.00, 7.00 and 10.00×10^{-2} mg/ml of pyridostigmine bromide was used. Each standard was injected six times.

THMP A seven-point standard curve was based on 0.16, 0.32, 0.64 1.28, 2.57, 5.15 and 10.30×10^{-3} mg/ml solutions. Each standard was injected six times.

All the standards were diluted with the mobile phase.

Samples

The samples consisted of pyridostigmine tablets (Solvay Duphar B.V., Amsterdam, The Netherlands). The declared content of pyridostigmine bromide is 30 mg (+5% overage) per tablet. The tablets are distributed in blister packs wrapped in an air-tight and light-protecting aluminum foil. A total of nine blister packs from a single batch number were analysed. Five of these originated from a depot in Norway, while the remaining four packages were collected from a portion of a batch which was sent with a Norwegian medical company to Saudi Arabia during the Gulf war in 1991. These samples were stored for about 4 months in the theatre and returned to Norway.

Chemicals

Acetonitrile HPLC grade S was obtained from Rathburn Chemicals Ltd (Walkerburn, U.K.), 1-hexanesulfonic acid sodium salt from Sigma Chemical Co. (St. Louis, U.S.A.), pyridostigmine from F. Hoffmann-La Roche Co. AG (Basel, Switzerland), THMP from Solvay Duphar B.V. (Amsterdam, The Netherlands) and sulfuric acid from NMD (Oslo, Norway).

Equipment

HPLC A Shimadzu LC6A (Kyoto, Japan) liquid chromatograph consisting of a single-headed

TABLE 1

Accuracy of the HPLC assay for pyridostigmine bromide

Amount injected (μg)	Accuracy [a] (%)
1.000	−0.23
1.802	1.01
3.020	0.53
4.206	−0.07
6.010	−0.78

[a] Accuracy (%) $100(\bar{x} - \mu)/\mu$
(μ, taken amount; $\bar{x}$, found amount).

TABLE 2

Accuracy of the HPLC assay for THMP

Amount injected (μg)	Accuracy [a] (%)
0.009	0.25
0.019	−1.78
0.038	−0.85
0.077	1.75
0.154	1.25
0.309	−0.37
0.618	0.90

[a] Accuracy (%) $= 100(\bar{x} - \mu)/\mu$
(μ, taken amount; $\bar{x}$, found amount).

pump and autosampler with controller unit was used. The detector, an LC-235 Diode Array Detector (Perkin Elmer, CT, U.S.A.) was operated at 270 nm. The column was connected to an oven (Model 480 with a controller, Kontron Instruments, Zürich, Switzerland) and the temperature was set at 35°C. The samples were chromatographed on a polymeric column, PRP-MP, 100 × 4.6 mm, 10 μm particles (Brownlee Labs. Inc., Santa Clara, U.S.A.) connected with a polymeric precolumn, PRP-GU, 30 × 4.6 mm, 10 μm particles (Brownlee Labs. Inc.). The mobile phase consisted of acetonitrile-water (7.8:92.2, v/v) mixture with 0.015 M hexanesulfonic acid sodium salt. The pH in the mobile phase was adjusted to 2.6 with 1 M H_2SO_4. The injection volume was 60 μl and the flow rate was 1 ml/min.

The chromatograms were integrated with The Vax Multichrom data aquisition system (VG Laboratory Systems Ltd, Cheshire, U.K.) using an external standard method. The data were recorded on a Digital MicroVAX 3100.

Calculation This was carried out on an Olivetti M40 computer with software developed in our laboratory. The system used a weighted regression line in computing the results.

Results and Discussion

Calibration graphs were constructed of peak height vs amount injected. Both calibrations graphs were linear in the range investigated: 1.0–6.0 μg corresponding to 6.7–40.0 mg/tablet for pyridostigmine bromide and 0.01–0.62 μg corresponding to 0.2–13.1% degradation for THMP. Standards calculated as samples were used as a measure of accuracy (Tables 1 and 2).

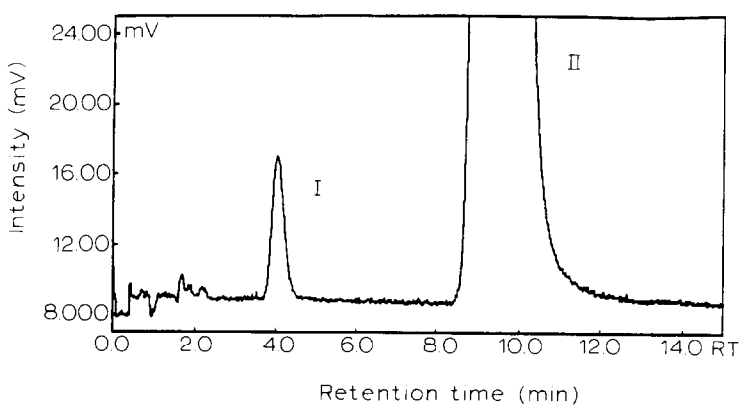

Fig. 2. Chromatogram of a sample. (I) 3-Hydroxy-1-methylpyridinium bromide; (II) pyridostigmine bromide.

TABLE 3

Reproducibility of a complete analysis (sample preparation and injection) and average content of PYR [a] and THMP

Store	RSD [b] of PYR	Average (mg/tablet)	RSD of THMP	Average (mg/tablet)
Norway	0.75	30.93	1.90	0.3764
Norway and SA [c]	0.68	31.07	2.41	0.3169

[a] Pyridostigmine bromide.
[b] Relative standard deviation of a complete analysis.
[c] Saudi Arabia.

Reproducibility was calculated by an analysis of variance. The chromatographic reproducibility of an injection within a subsample for pyridostigmine was 0.2% (54 df). The relative standard deviation of a complete analysis (sample preparation and injection) was calculated for both pyridostigmine bromide and THMP (Table 3).

The method was used to determine the degradation of pyridostigmine tablets stored under two different conditions. Fig. 2 shows a chromatogram of a representative sample. The samples stored only in Norway showed degradation of 1.6%. Samples stored in both Saudi Arabia and Norway showed 1.4% degradation. This difference in degradation is small, but was found to be significant. It appears that the stay in Saudi Arabia had slowed the degradation of pyridostigmine in the tablets. This difference may reflect the fact that both temperature and humidity in the depots varied. The fall in temperature during the night in Saudi Arabia was considerably greater than in the store in Norway, where the temperature was constant and within 20–22°C.

Conclusions

A liquid chromatographic method for determination of pyridostigmine and its major degradation product in tablets has been proposed. The sample procedure is easy to perform and rapid, with a chromatographic analysis time of 15 min. The use of pyridostigmine as a prophylactic against nerve gas poisoning has emphasized the need for a suitable and reliable method of analysis. The proposed method has been applied to analysis of the degradation of pyridostigmine in tablets stored under two different conditions. It shows the usefulness of this procedure in routine control as well as accelerated studies of such tablets.

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