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Short Communication

Determination of phenylmercuric nitrate in pharmaceuticals by HPLC

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Introduction

Phenylmercuric nitrate (PMN) is commonly used in pharmaceutical preparations as an antimicrobial preservative in a concentration of 10–20 mg l^{-1}. Few methods have been reported for the analysis of organomercurial compounds. Although atomic absorption spectrophotometry has been applied [1], high-performance liquid chromatographic methods with UV detection have been described as more specific; these methods involve the analysis of PMN after chloroform extraction [2] or after chelation of organic mercury with substituted dithiocarbamates [3, 4]. In particular, the chelation of PMN by morpholinedithiocarbamate was developed by Parkin [4]; the reaction was shown to be rapid and to result in the formation of a compound with a suitable molar absorptivity in the UV range.

The present study was undertaken to validate a rapid and sensitive method for the direct determination of PMN in a nasal pharmaceutical formulation. Since it has been shown that organomercurial compounds may be sorbed into plastics [1, 5] the possible loss of PMN from a nasal solution packed in glass or plastic containers was assessed in this study.

Experimental

Chemicals

Phenylmercuric nitrate, neomycin sulphate and morpholine were purchased from Merck (Darmstadt, Germany) and all other chemicals were analytical grade (Merck).

Preparation of complexing reagent

Morpholinedithiocarbamate (MDTC) was synthesized as reported previously by Parkin [4]. A solution of MDTC (1 g l^{-1}) was prepared in water–acetonitrile (1:1, v/v). This solution should be used within 8 h of preparation.

Standard solution of phenylmercuric nitrate

A solution of phenylmercuric nitrate (20 mg l^{-1}) was prepared and transferred into a glass flask.

Apparatus

The experiments were performed with a Varian system comprising a 5000 liquid chromatograph equipped with a Rheodyne valve (10-μl loop), a model UV-100 absorbance detector and a CDS 401 integrator (Varian, Palo Alto, CA, USA).

Chromatographic conditions. The separation was accomplished on a 250 × 4 mm i.d. column packed with 5-μm Lichrospher 100 RP-18 (Merck) used with a Lichrocart 4-4 100 RP18 (5-μm) precolumn (Merck). The mobile phase was water–acetonitrile–0.3 mM EDTA (70:25:5, v/v/v). A flow rate of 1 ml min^{-1} was used under a resultant pressure at 90 atm. The analytical wavelength for peak detection was set at 258 nm.

Liquid chromatographic analysis. Derivatization was achieved by vigorously mixing 1 ml of MDTC solution with 1 ml of the standard solution or formulation samples; chelation was effective after 30 min in the dark. A 10 μl

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volume was then injected into the chromatograph.

Preparation of a nasal formulation

A solution equivalent to a nasal formulation was prepared to the following formula: neomycin sulphate 1.25 g, phenylmercuric nitrate 0.002 g, sodium citrate 0.025 g, citric acid 0.025 g and isotonic saline to 100 g (pH 6.8). This formulation was directly analysed for PMN. Aliquots (15 ml) of the preparation were placed in 30-ml bottles each fitted with a Teflon closure; three aliquots were placed in type II glass bottles and three in polyethylene bottles. These containers were kept in the dark at room temperature (19–22°C) throughout the experiment. To evaluate the effect of long storage, samples of solutions in each type of container were analysed for PMN immediately and after 3 and 9 months.

Results and Discussion

A chromatogram of the MDTC–PMN complex is shown in Fig. 1. The retention time was 5.3 min.

Validation

The linearity of the calibration graph was checked by assaying different dilutions of the PMN standard solution in the range 0.1–100 mg ml^{-1}. The linear regression equation was: $y = 0.61x - 0.095$ ($r = 0.9999$), where y is the peak area (arbitrary units) and x is the concentration (mg l^{-1}).

The lower limit of detection was 0.03 mg l^{-1}. The precision was evaluated for a solution of PMN (20 mg l^{-1}). The relative standard deviations were 1.4% for repeatability ($n = 10$) and 2.1% for reproducibility ($n = 5$). The reproducibility was also evaluated for the analysis of PMN in the nasal formulation; the relative standard deviation was 3% ($n = 5$).

Specificity

No interference was observed between the MDTC–PMN peak and peaks resulting from an excess of MDTC or from other formulation components. Since it has been reported that phenylmercury salts can be degraded to inorganic mercury [6], the method was performed with Hg^{+} nitrate and Hg^{2+} nitrate (10 mg l^{-1}) in the mixture; no problems were encountered in the presence of these salts. The results show that the method has satisfactory precision and sensitivity and that the use of a great excess of chelation reagent allowed the assay of a large range of PMN concentrations without modifying the protocol.

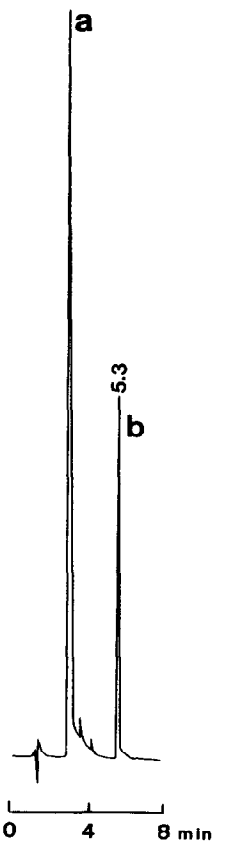

Figure 1
Chromatogram of the PMN assay in a nasal pharmaceutical formulation. (a) Excess of MDTC; (b) MDTC–PMN complex.

Table 1
Residual PMN concentration (%) of the nasal formulation packed in two different types of containers

Storage time (months)	Glass containers ($n = 3$)	Polyethylene containers ($n = 3$)
0	100*	100*
3	100	27
9	94	25

*The value 100% was assigned to the mean concentration of the three containers of each type determined at the start of the experiment.

Analysis of the nasal formulation

The results are presented in Table 1. A slight decrease in the PMN concentration of the solution in the glass bottles was observed only after 9 months whereas a decrease in PMN

concentration in the solutions kept in polyethylene bottles was noticeable from the third month. This result cannot be attributed to the degradation of PMN but clearly shows that PMN is sorbed into the plastic. The stabilization of the PMN level in the plastic bottle between the third and sixth month could indicate saturation of the plastic materials.

Conclusions

This HPLC technique provides a rapid and simple method for the quantitative analysis of PMN in a complex mixture to determine the loss of the preservative, which might be hazardous to patients by allowing microbial growth in formulated solutions.

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