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Simultaneous determination of methylparaben, propylparaben and thimerosal by high-performance liquid chromatography and electrochemical detection

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Abstract

A reversed-phase high-performance liquid chromatographic method using amperometric detection has been developed for the analysis of methylparaben, propylparaben and thimerosal. The liquid chromatographic separation of the three preservatives was made possible on a C_{18}-bonded silica column with a mixed solvent consisting of methanol and aqueous 0.02 M phosphoric acid (59:41, v/v). A potential value of +1.25 V versus Ag/AgCl was chosen for simultaneous analysis. The limits of detection were 1, 2 and 5 ng for a 20 μl injection volume of methylparaben, propylparaben and thimerosal, respectively. The analysis time of less than 20 min in this study was found to be applicable for routine analysis of these compounds in pharmaceutical products. © 1997 Elsevier Science B.V.

Keywords: Methylparaben; Propylparaben; Thimerosal; HPLC; Electrochemical detection; Simultaneous determination

1. Introduction

Recently, preservatives in consumer products have received keen attention because of their possible side-effects on humans. As a result, a fast, simple and accurate method of analysis was necessary. Methylparaben (MP), propylparaben (PP) and thimerosal (TMS) are effective antibacterial and antifungal agents which are commonly used as preservatives in foods, beverages, cosmetics and pharmaceuticals. An HPLC analysis of MP and PP in the mixture was made by the UV absorption method with C_8-, $C_{18}-$ and CN− columns [1–7] but the method was not able to detect a concentration level of μg ml^{-1} with sufficient sensitivity and selectivity. Various analytical methods for TMS have been developed, such as spectrophotometry [8,9], atomic absorption spectrometry [10,11] and polarography [12,13].

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The decomposition of TMS in aqueous solution was first recognized by Reader [14]. He showed that the decomposition of thimerosal in water to thiosalicylic acid and ethylmercuric hydroxide was a reversible reaction. Since then, HPLC methods were utilized for the selective determination of TMS and its decomposed products in sample mixtures but, generally, the method was not sensitive enough (>5 μg ml^{-1}), and it lacked the simultaneous determination of MP, PP and TMS.

An LC/ECD (electrochemical detector) assay for TMS was reported by Jesus et al. [15,16]. It was based on the analysis of TMS and its two main degradation products, thiosalicylic acid and dithiodibenzoic acid. But this method was not designed as a simultaneous determination method for MP, PP and TMS.

In this study we report a development of an HPLC/ECD method with high sensitivity, which is suitable for the simultaneous determination of MP, PP and TMS in pharmaceutical products.

2. Experimental

2.1. Apparatus

The high-performance liquid chromatograph used in this study was a Vintage 2000 LC (Orom Tech, Seoul, South Korea) with an HP 3390 integrator (Hewlett-Packard, Palo Alto, CA) and a UV-200 variable wavelength UV/VIS detector (Scientific Systems, State College, PA). An amperometric electrochemical detector (EG & G, Princeton Applied Research Corp., model 400, Princeton, NJ) was also used. The EC detector was a thin-layer type which consisted of a rectangular channel equipped with a glassy carbon working electrode (electrode area 0.07 cm^2, cell volume 4 μl). A stainless-steel counter electrode block was located at the opposite side of the working electrode and an Ag/AgCl reference electrode was placed at the down stream of the flow as shown in Fig. 1. The outlet of the UV/VIS detector was connected to the inlet of the ECD. A Bioanalytical System BAS 100 B/W electrochemical analyzer (W. Lafayette, IN) was used for cyclic voltammetry of MP, PP and TMS.

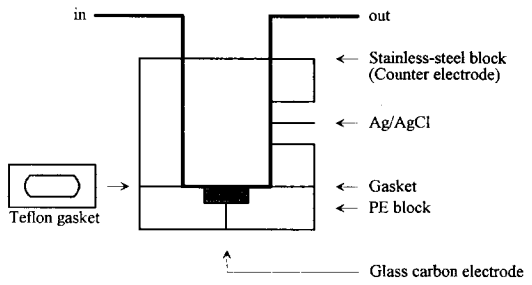

Fig. 1. Structure of the electrochemical thin layer cell.

2.2. Reagents

MP, PP and TMS (Fig. 2) were obtained from Aldrich Chemical (Milwaukee, WI). The mobile phase was made of HPLC grade methanol, reagent grade phosphoric acid and sodium phosphate dibasic. Other chemicals used were analytical reagent grade, and they were used without further purification. HPLC grade methanol and water which was purified with a Nano-pure II purification system (Barnsted, Newton, MA) were used to make solutions.

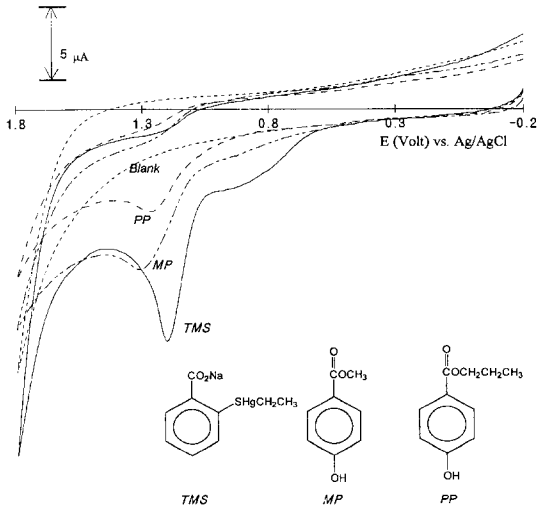

Fig. 2. Cyclic voltammograms of MP, PP and TMS. Concentration, 50 μg ml^{-1} in methanol 0.02 M aqueous phosphoric acid (59:41, v/v). Scan rate, 100 mV s^{-1}.

2.3. Electrochemistry

The applied potential was chosen from the cyclic voltammograms shown in Fig. 2. The voltammograms were obtained using a glassy carbon working electrode, a platinum counter electrode, and an Ag/AgCl reference electrode in the mixture of methanol and aqueous 0.02 M phosphoric acid (59:41, v/v). Potentials ranging from +1.10– +1.30 V versus Ag/AgCl were applied to the electrochemical thin layer cell (Fig. 1) in order to determine the optimum potential for simultaneous detection.

2.4. Chromatography

In order to find out the proper mobile phase, phosphate buffers were prepared with the concentration range of 0.01–0.03 M of phosphoric acid and the pH were adjusted with a 0.5 M sodium phosphate dibasic solution. The composition and pH of the mobile phase were varied based on the retention time (< 20 min), peak sharpness and resolution. To minimize random electrical noise from the electrochemical detector the mobile phase was degassed by filtering through a 0.45 μm membrane filter.

Several C_{18}-columns of μ-Bondapak C_{18} (Waters, Milford, MA), Spherisorb ODS-2 (Sigma-Aldrich, Milwaukee, WI), LiChrosorb RP-18 (Merck, Darmstadt, Germany), Capcell Pak C_{18} (Shiseido, Tokyo, Japan) and Rexchrom ODS (Regis, Morton Grove, IL) were also examined to find out if there is any difference in terms of separation and response.

2.5. Determination of MP, PP and TMS in pharmaceuticals

To determine the contents of MP, PP and TMS in pharmaceutical products with the HPLC/ECD, two methods of sample preparation were employed. In the case of cream or ointment they were extracted with a 50% aqueous methanol solution. The solution extract was filtered through a 0.45 μm membrane filter and injected into the chromatographic system. In the case of ophthalmic solutions they were filtered and directly injected with no pretreatment.

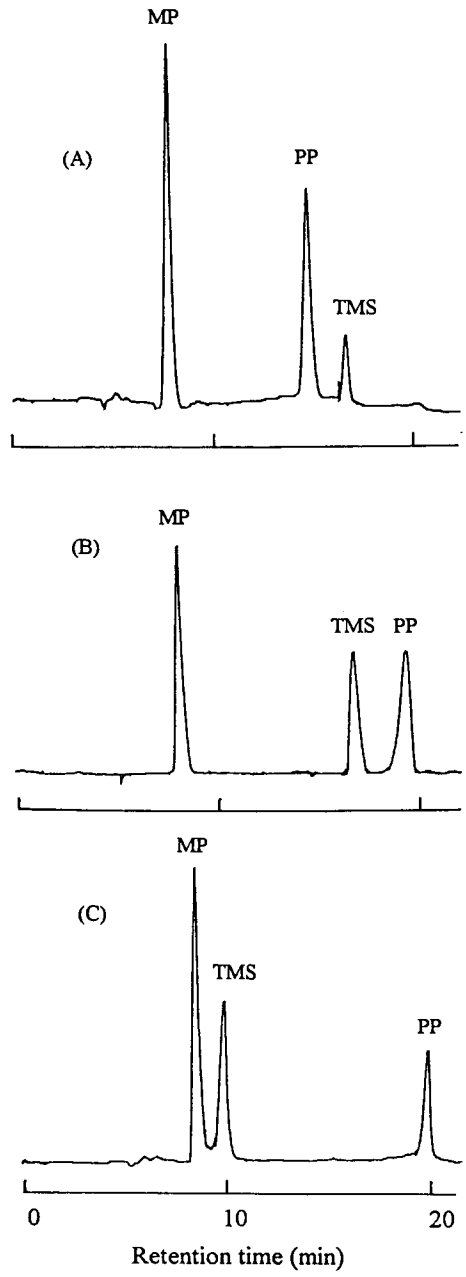

Fig. 3. Chromatograms obtained with standards containing MP, PP and TMS at different pH. (A) 2.5, (B) 4.5 and (C) 6.0 in the mobile phase. Column, Capcell Pak C_{18}. Mobile phase, methanol 0.02 M aqueous phosphoric acid (59:41, v/v). Concentration, MP, PP, 1.0 μg ml^{-1} and TMS, 2.0 μg ml^{-1}. Flow rate, 1.0 ml min^{-1}. Detector, amperometric detection on a glassy carbon electrode at +1.25 V vs. Ag/AgCl. Amount injected, 10 μl.

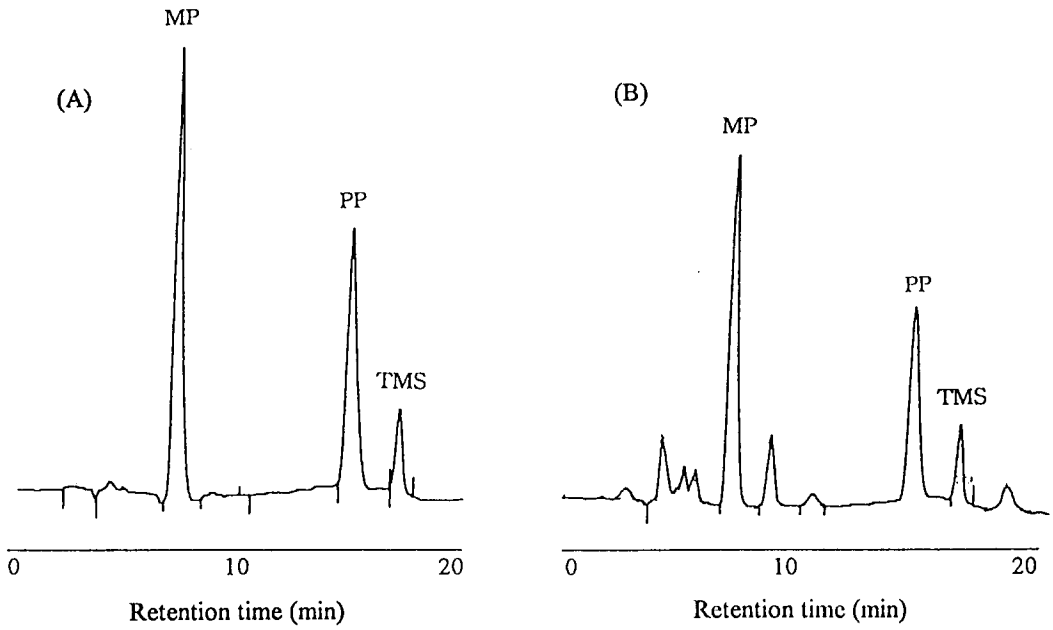

Fig. 4. Effect of detection system in chromatograms obtained with standards containing MP, PP and TMS. (A) Amperometric detection on a glassy carbon electrode at +1.25 V vs. Ag/AgCl. (B) UV at 210 nm. Amount injected, 10 μl. Concentration, MP, PP, 2.5 μg ml^{-1} and TMS, 5.0 μg ml^{-1}. Column, Capacell Pak C_{18}. Mobile phase, methanol 0.02 M aqueous phosphoric acid (59:41, v/v).

3. Results and discussion

3.1. Electrochemical study

The electrochemical properties of MP, PP and TMS were investigated by cyclic voltammetry in order to find out the possibility of simultaneous determination. The cyclic voltammograms in Fig. 2 were obtained at a concentration of 50 μg ml^{-1} in a mixed solvent of methanol and 0.02 M phosphoric acid (59:41, v/v), which is identical to the mobile phase used in the separation. For MP and PP, the voltammograms show a similar anodic wave at +1.30 and +1.27 V versus Ag/AgCl, respectively. They are due to oxidation of the phenolic moiety in the compound. TMS produces two anodic peaks, one at +1.20 V and a shoulder peak at about +0.9 V. The broad shoulder peak is due to the oxidation of thiosalicyclic acid, one of the decomposition products of TMS as reported by Jesus et al. [15]. Accordingly, we have chosen a more positive potential than +1.10 V for simultaneous detection of the three compounds. At a range of potentials from +1.10–+1.30 V versus Ag/AgCl, the response increases with increasing applied potential. Similar behavior is observed at potentials above +1.30 V, the noise level of the blank solution, however, increases as well. In order to optimize the response signal while reducing the noise level, a suitable potential of +1.25 V was chosen for analysis.

3.2. Chromatography

The pH of the aqueous mobile phase is adjusted in the range 2.0–5.0 for optimal separation of the compounds because a slight pH difference of the mobile phase can affect the resolution and elution time. When the pH increases the retention time of TMS markedly decreases whereas those of MP and PP increases as shown in Fig. 3. Increasing the volume ratio of aqueous 0.02 M phosphoric acid to methanol in the mobile phase increases both resolution and retention time. At pH < 2.0, the stability of the column seems uncertain; at pH > 5.0, the limiting current of TMS decreases

when pH increases. We have selected a pH of 2.5 considering the elution time, resolution, stability of column seems uncertain; at pH > 5.0, the limiting current of TMS decreases when pH increases. We have selected a pH of 2.5 considering the elution time, resolution, stability of the column and the size of limiting current.

For comparison of several columns, elution time and resolution proves the Capcell Pak C_{18} (Type UG 120 A, 5 μm, 250 mm × 4.6 mm i.d.) and Rexchrom ODS (100 A, 5 μm, 250 mm × 4.6 mm i.d.) are suitable for the simultaneous determination method of this study.

Chromatograms in Fig. 4 were obtained with a standard solution containing MP (2.5 μg ml^{-1}), PP (2.5 μg ml^{-1}) and TMS (5 μg ml^{-1}). The performance of ECD and UV/VIS detectors is directly compared with chromatograms obtained under the same separation conditions. The UV detector produces a poorer signal than ECD and several impurities are observed. The chemical nature of these impurities are beyond the scope of this study and are not identified. However, the chromatograms obtained with the ECD are free of impurities encountered with the UV/VIS detector.

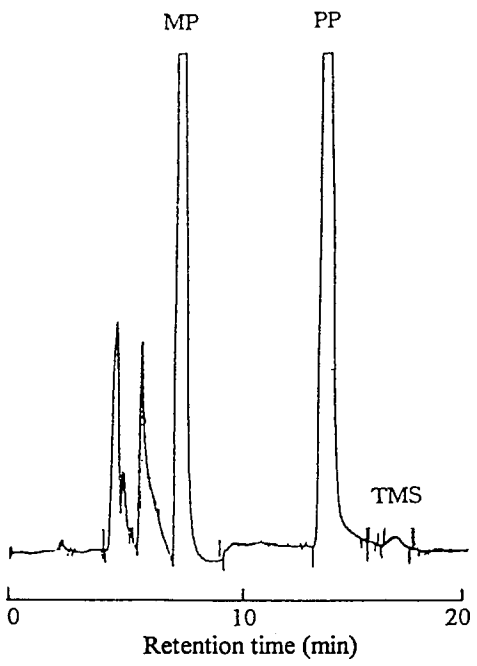

Fig. 5. Typical chromatogram obtained with a pharmaceutical sample. Amount injected, 10 μl. Column, Capcell Pak C_{18}. Mobile phase, methanol 0.02 M aqueous phosphoric acid (59:41, v/v). Flow rate, 1.0 ml min^{-1}. Detector, amperometric detection on a glassy carbon electrode at +1.25 V vs. Ag/AgCl.

The detection limits for a 20 μl injection of MP, PP and TMS are 1, 2 and 5 ng (at signal:noise of 3), respectively. In addition, very high correlation coefficients of 0.9996, 0.9994 and 0.9983 are obtained for MP, PP and TMS, respectively.

3.3. Determination of MP, PP and TMS in pharmaceutical products

The chromatogram in Fig. 5 was obtained using the HPLC/ECD method with a real sample (ophthalmic solution, H. Pharmaceutical, Seoul, South Korea). A 100 ml sample containing 20.2 mg MP, 10.0 mg PP and 0.1 mg TMS was filtered through a 0.45 μm membrane filter before direct injection into the HPLC/ECD system. The average amount of MP, PP and TMS determined was 100.1 ± 0.2, 99.5 ± 0.1 and 98.7 ± 0.2% of the actual amount, respectively.

In order to obtain a sizable TMS peak, a large amount of MP and PP has to be injected and the chromatogram produces two peaks with short retention times before the elution of MP. It is evident that other minor impurities are also present in the sample. The chromatograms clearly show the high specificity and dynamic range of the electrochemical detector system. No pretreatment of the sample is necessary even though the concentration of MP is around 200 times that of TMS. With the UV detector, however, it is not possible to detect all three components unless a programmed gain amplifier is employed in the system.

4. Conclusions

Our study on the simultaneous determination of MP, PP and TMS has shown that the three compounds can be separated and determined by an HPLC/ECD at +1.25 V versus Ag/AgCl

within 20 min. The method is able to detect a concentration level of ng ml^{-1} with high selectivity. Its application to pharmaceutical products provides a fast, simple, and accurate method for routine quality control of commercial products containing MP, PP and TMS.

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