Journal of Chromatography, 166 (1978) 316–319

CHROM. 11,239

Note

Determination of methyl *p*-hydroxybenzoate and propyl *p*-hydroxybenzoate in an anticholinergic antidote by ion-pair high-performance liquid chromatography

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(Received June 5th, 1978)

Various methods for assaying methyl *p*-hydroxybenzoate and propyl *p*-hydroxybenzoate have been reported[1–3]. Fitzpatrick *et al.*[4] described a high-performance liquid chromatographic (HPLC) method for measuring the concentration of these two parabens in cosmetics. Sokol[5] and Lachman *et al.*[6] have shown that the quantification of methyl *p*-hydroxybenzoate is possible using colorimetric and spectrophotometric procedures. At the same time, a gas chromatographic method has been employed as a standard analytical technique for quantitating parabens in various therapeutic drugs[7].

Of the many methods used for the analysis of methyl *p*-hydroxybenzoate and propyl *p*-hydroxybenzoate, few of them offer the advantages of ion-pair HPLC. Current methods ulitize procedures which are arduous and time consuming. Sometimes, specificity is lacking. Because of these inadequacies, a relatively simple and specific ion-pair HPLC method is described for separation and quantitating methyl and propyl paraben in a multicomponent anticholinergic antidote. The procedure is also applicable for assaying *p*-hydroxybenzoic acid. No solvent extraction or pre-treatment is required prior to the analysis. Concentrations as low as 1 ng on column are assayable by the method. Total analysis time is 9 min per sample. The method is used to monitor the stability of methyl and propyl *p*-hydroxybenzoate in the anticholinergic drug formulation during its manufacture and later, during storage.

MATERIALS AND METHODS*

Apparatus

A Waters Assoc. Milford, Mass., U.S.A. Model ALC/GPC-204 liquid chromatograph was used throughout this study. The system consisted of two Model 6000A high-pressure pumps, a Model 660 solvent programmer, a U6K loop injector, a 254 nm UV detector, a Houston Instrument Omni-Scribe A 5000 dual-pen recorder and a Columbia Scientific Supergrator-3 integrator.

* The manufacturers' names and products are given as scientific information only and do not constitute an endorsement by the United States Government.

Reagents

Spectroquality acetonitrile (Eastman-Kodak, Rochester, N.Y., U.S.A.) mixed with PIC-B7 reagent (1-heptane sulfonic acid, Waters Assoc.) was used as the mobile phase. Methyl *p*-hydroxybenzoate, propyl *p*-hydroxybenzoate (U.S. Pharmacopeial Convention, Rockville, Md., U.S.A.), *p*-hydroxybenzoic acid and sulfanilic acid (Aldrich, Milwaukee, Wisc., U.S.A.) were used to prepare all working standards. A commercially prepared multicomponent anticholinergic antidote (Cartrix parenteral systems) was analyzed for its methyl and propyl *p*-hydroxybenzoate concentrations. Sulfanilic acid was incorporated into the mixture for maintaining quality control.

Procedure

A pre-packed 30 cm × 3.9 mm reversed-phase μBondapak C_{18} column (Waters Assoc.) was used to separate all compounds in the standard and experimental samples. A stock standard solution, containing 1 μg/μl of methyl *p*-hydroxybenzoate, propyl *p*-hydroxybenzoate, and *p*-hydroxybenzoic acid was prepared in 0.002 *N* HCl. The commercially prepared antidote was diluted (1:10) with 0.002 *N* HCl. Sulfanilic acid (1.05 μg/μl) was incorporated into this mixture as an internal standard.

The mobile phase consisted of 0.01 *M* 1-heptanesulfonic acid and acetonitrile. The PIC reagent was prepared by mixing 20 ml of the pre-package solution with 480 ml of glass distilled water. The pH of the reagent was 3.40.

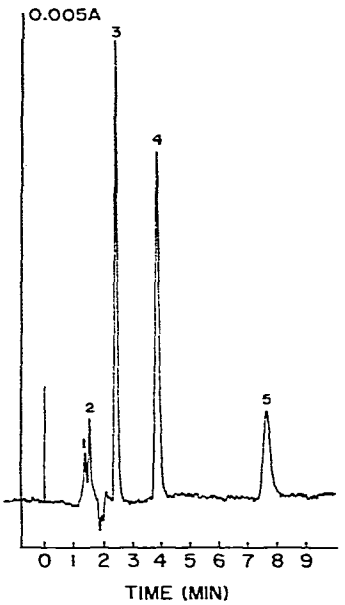
Employing the dual pumping HPLC system, along with the 660 solvent programmer, a 35–65% mixture of acetonitrile to PIC reagent was isocratically pumped through the column. The flow-rate was 1.5 ml/min. All separations were carried out at ambient temperatures. Volumes of 2 ml were introduced into the column through a continuous-flow loop injector. Peak areas were measured by an on-line computing integrator.

RESULTS AND DISCUSSION

Although the main thrust of our previous studies has been directed primarily towards the stability and purity of the prime constituents of this anticholinergic antidote, namely N,N′-trimethylene-bis-(pyridinium-4-aldoxime) dibromide (TMB-4), atropine and benactyzine, much work has been done on the ancillary products of this mixture. Included within this group are methyl and propyl *p*-hydroxybenzoate and their hydrolytic degradation product, *p*-hydroxybenzoic acid. The monitoring of the concentration of these compounds in this parenteral survival kit is of the utmost importance. Maintaining a therapeutic agent, free of active bacteria and fungi depends greatly upon the bacteriostatic and fungistatic actions of these two compounds.

With the development of this analytical method, a simpler and more specific procedure is available for quantitating the concentration of methyl and propyl paraben. Utilizing this technique, a series of standards and experimental samples were analyzed. The chromatogram shown in Fig. 1, depicts the separation and resolution of *p*-hydroxybenzoic acid, methyl *p*-hydroxybenzoate and propyl *p*-hydroxybenzoate in standard solution. For a series of standard solutions containing the three compounds, linearity was observed for all concentrations measured (1–1000 ng).

The applicability of the method is further demonstrated by the chromatogram



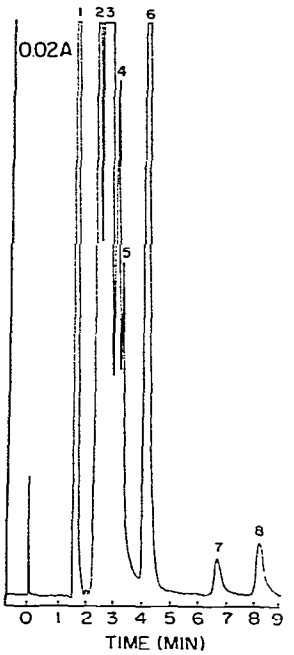


Fig. 1. Separation of a standard solution, containing (1) unknown, (2) unknown, (3) *p*-hydroxybenzoic acid, (4) methyl *p*-hydroxybenzoate and (5) propyl *p*-hydroxybenzoate. Column: 3.9 mm × 30 cm μBondapak C_{18}. Mobile phase: acetonitrile–1-heptane sulfonic acid (35:65).

Fig. 2. Separation of a commercially prepared anticholinergic antidote containing (1) sulfanilic acid (internal std.), (2) 4-pyridine aldoxime, (3) N,N′-trimethylene-bis-(pyridinium-4-aldoxime) dibromide (4) atropine sulfate + unknown, (5) unknown, (6) methyl *p*-hydroxybenzoate, (7) benactyzine hydrochloride and (8) propyl *p*-hydroxybenzoate. Column temperature: ambient; mobile phase: acetonitrile–1-heptanesulfonic acid (35:65); flow-rate: 1.5 ml/min.

TABLE I

PRECISION AND ACCURACY OF ION-PAIR HPLC METHOD FOR ASSAYING METHYL AND PROPYL *p*-HYDROXYBENZOATES IN AN ANTICHOLINERGIC ANTIDOTE

	Theoretical value (ng/μl)	*Assayed value*	
		Mean (ng/μl) ± S.D.	*Coefficient of variation (%)*
Methyl paraben	50	49 ± 0.8	1.83
Propyl paraben	5	4.9 ± 0.03	2.40

shown in Fig. 2. Methyl and propyl *p*-hydroxybenzoate are clearly discernible from the other components contained in the mixed formulation.

Results shown in Table I, summarize the data obtained from the separation of 20 anticholinergic drug samples.

The accuracy and precision of the method are excellent. The concentrations of methyl *p*-hydroxybenzoate and propyl *p*-hydroxybenzoate in the experimental samples compared favorable with the theoretical values of the drug formulation.

In summary this method offers an improved analytical technique for separating and quantitating methyl and propyl paraben in simple and complex mixtures.

ACKNOWLEDGEMENTS

We thank Ms. Margot M. Stevens for her excellent secretarial assistance and Dr. Gale E. Demaree for his enthusiastic support during this study.

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