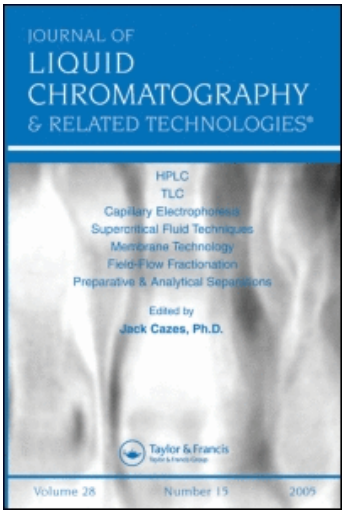

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

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Daryl A. Roston[a]
[a] Searle Research and Development, Illinois

To cite this Article Roston, Daryl A.(1987) 'HPLC Method Development for a New Antiarrhythmic Drug', Journal of Liquid Chromatography & Related Technologies, 10: 15, 3427 — 3440
To link to this Article: DOI: 10.1080/01483918708081881
URL: http://dx.doi.org/10.1080/01483918708081881

JOURNAL OF LIQUID CHROMATOGRAPHY, 10(15), 3427-3440 (1987)

HPLC METHOD DEVELOPMENT FOR A NEW ANTIARRHYTHMIC DRUG

Daryl A. Roston
Searle Research and Development
4901 Searle Parkway
Skokie, Illinois 60077

ABSTRACT

The present report concerns HPLC method development for chemical samples of a new antiarrhythmic drug 2-methyl-5-phenyl-5-[2-[2-N,N-bis (1-methylethyl amino]ethyl]-1,3 diazabicyclo[4.4.0]octen-4-one (MPMED). Selectivity optimization for MPMED and several synthetic process intermediates with reversed-phase HPLC conditions is described. Also, the use of electrochemical and UV absorption detection for MPMED samples has been evaluated. The developed method has been validated for generation of assay data for chemical lots of MPMED.

INTRODUCTION

Numerous communications have described method development and applications for high-performance liquid chromatographic (HPLC) analysis of antiarrhythmic drugs in matrices such as plasma and urine

(1-8, the cited references are representative of an extensive field.). The present study concerns HPLC method development for chemical samples of a new antiarrhythmic drug, 2-methyl-5-phenyl-5-[2-[2-N, N-bis(1-methylethyl amino]ethyl]-1,3 diazabicyclo [4.4.0]octen-4-one (MPMED). The structure is shown in Figure 1A. The method development has been completed to provide a basis for generating quantitative data for chemical samples of MPMED. To ensure that the method has selectivity for MPMED, reversed-phase HPLC conditions have been optimized for resolution of MPMED and several intermediates in the MPMED synthetic process. Additional aspects of the method development described in the present report concern detection and method validation. Electrochemical detection and UV absorption detection have been evaluated for use during HPLC analysis of MPMED samples. Also, the developed method has been validated for generation of quantitative data for MPMED lots with external standards.

EXPERIMENTAL

Reagents and Materials

MPMED samples and related compounds were provided by the Chemical Development Department of Searle Research and Development. The MPMED reference

standard was purified by recrystallization. The purity of the standard was verified with several methods, including differential scanning calorimetry, thin-layer chromatography, and HPLC. Mobile phase constituents were as follows: HPLC Water, concentrated phosphoric acid, Baker Chemical Co.(Phillipsburg, N.J.); triethylamine (TEA), Aldrich Chem.Co. (St. Louis, MO.); acetonitrile, Mallinckrodt, (Paris, Kentucky).

Chromatography System

All experiments were completed with a Hewlett-Packard 1084B liquid chromatograph equipped with a Schoeffel Spectroflow Monitor 770 variable wavelength detector (Kratos Analytical; Ramsey, N.J.). The system was also equipped with an ESA Model 5100A Coulochem Detector (ESA, Inc.; Bedford, MA). The ESA electrochemical detector was equipped with a Model 5012 wall-jet, glassy-carbon detector cell and a Model 5020 guard cell. The guard cell potential was +1.0 volt. Peak height and peak area measurements were made with an in-house chromatographic data management system.

The aqueous portion of the mobile phase was prepared by adding 20.0 mL of TEA to 1800 mL of water phase and adjusting the pH with concentrated

phosphoric acid (TEAP). After the pH adjustment, the solution was diluted to volume in a 2.0 L volumetric flask. The mobile phase in the present study was 84% TEAP media and 16% acetonitrile (vol/vol). The mobile phase was filtered before use. A Supelco LC-18-DB (DB= deactivated for basic compounds) column was used for all experiments. (Supelco, Inc., Bellefonte, PA.) Other pertinent HPLC parameters were as follows: injection volume, 25 mcl; flow, 2.0 mL/min; column temperature, ambient; column dimensions, 250 mm X 4.6 mm i.d.; detection wavelength, 205 nm.

Sample Preparation / Quantitation

Standard solutions for calibration and quantitation were prepared by dilution of a 2.0 mg/ml MPMED stock solution. For quantitative analysis, sample and standard solutions were approximately 1.0 mg/ml. All MPMED solutions were prepared in TEAP/ACN mobile phase.

External standard quantitation was used to generate all quantitative data. The response factor (peak area/concentration) for the MPMED standard was used to calculate the experimental sample concentration. Percent purity values are the experimentally found concentration (mg/mL) normalized

to the actual concentration. During the generation of quantitative data, each set of two sample injections was bracketed by injections of the standard.

RESULTS AND DISCUSSION

Selectivity Optimization / Detection

Efforts were directed towards optimization of chromatography conditions and detection for the resolution of MPMED and the six potential impurities shown in Figure 1. Structures B-G are synthetic process intermediates, stereoisomers of the synthetic process intermediates, and the stereoisomer of MPMED. Initial efforts concerned improving peak assymetry for MPMED. It was found that the MPMED peak showed considerable tailing when acetonitrile-water mobile phase and conventional octadecylsilane columns were employed. To alleviate the tailing problem, the mobile phase was buffered with TEA and phosphoric acid. Although the use of TEA in the mobile phase improved peak symmetry, MPMED peak tailing was still observed with conventional octadecylsilane columns. Peak symmetry was further improved when a Supelco LC-18-DB column was used.

Due to the presence of amine functional groups in MPMED and the anticipated impurities, the retention of the compounds as a function of mobile phase pH was

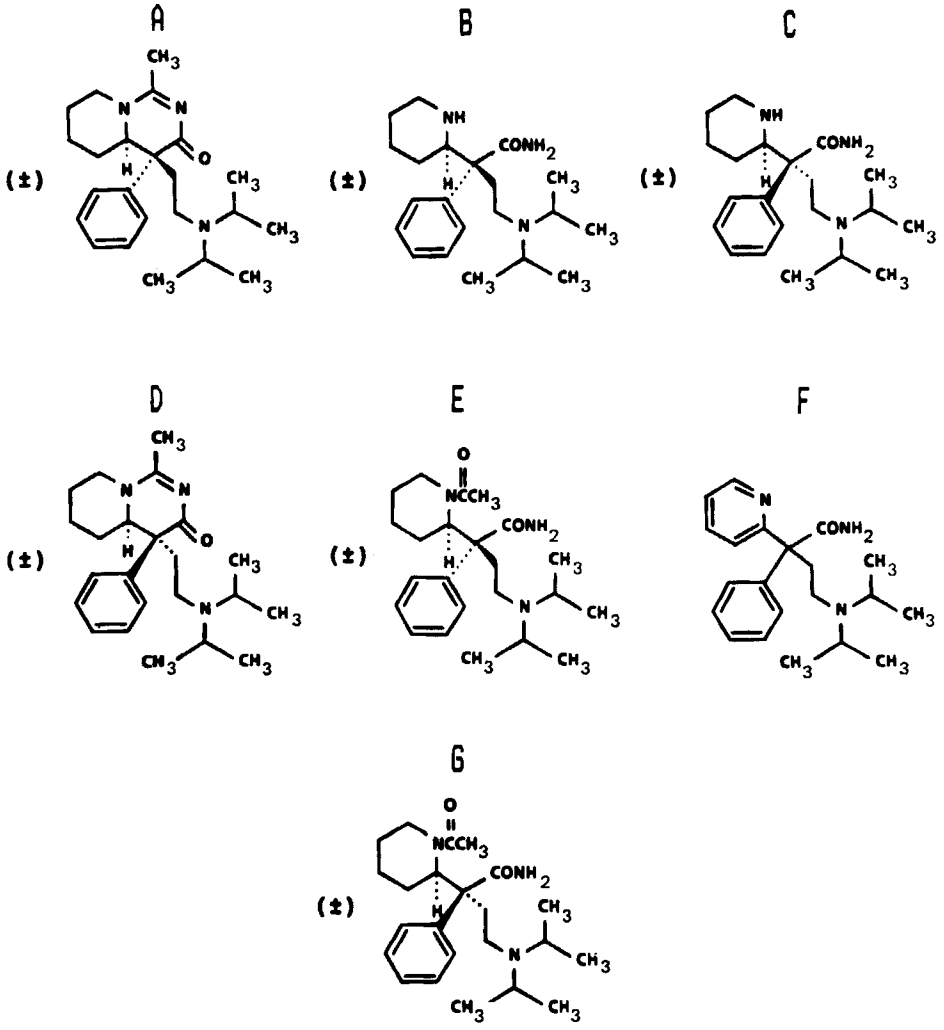

Figure 1. MPMED and anticipated synthetic process impurities.

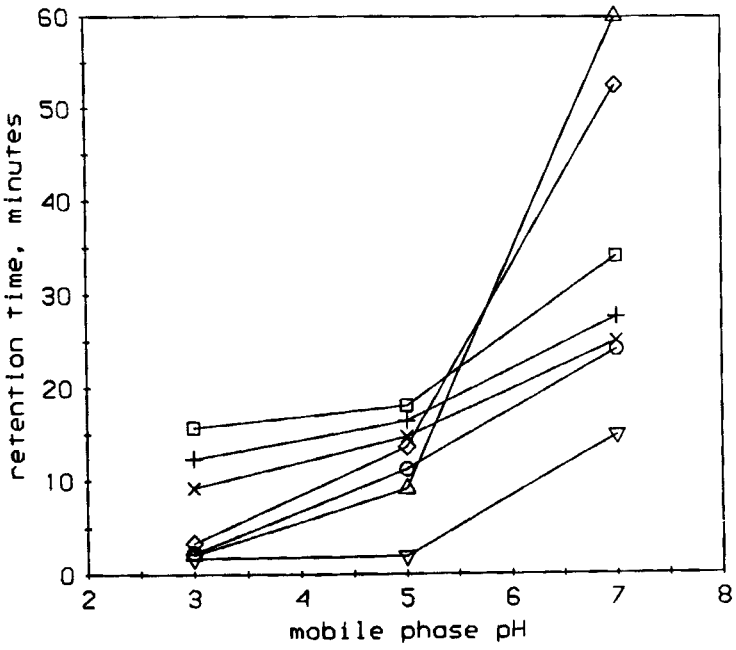

Figure 2. Retention vs. pH for MPMED and the anticipated process impurities. Conditions: mobile phase, 16% ACN, 84% 70 mM TEA adjusted to pH 3-7 with concentrated phosphoric acid; column, Supelco ODS-DB; column dimensions, 250 mm x 4.6 mm (i.d.); particle size, 5 micron; flow, 2.0 ml/min, O,MPMED; Δ,B; ∇,C; ◇,D; □,E; +,F; ×,G.

studied. A pH range of 3-7 in TEAP/ACN media was evaluated, based on pK values for MPMED of 4.6 and 9.8. Examination of the pH-retention data shown in Figure 2 revealed that the seven component mixture could be resolved with a mobile phase pH of 4-5. Figure 3 depicts the chromatogram recorded for a mixture of the compounds in Figure 1 when pH 4.5

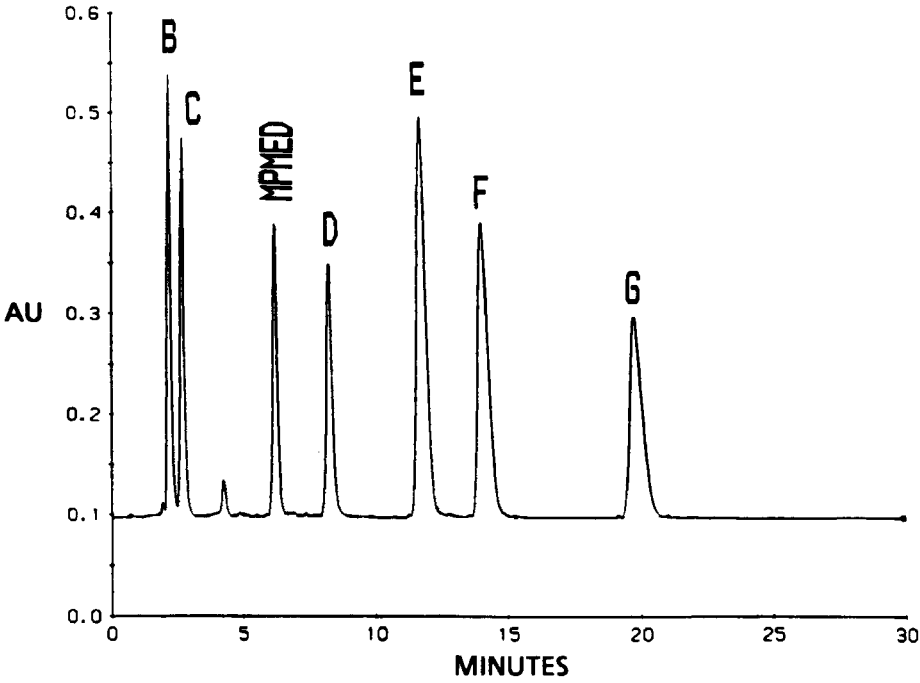

Figure 3. Chromatogram of MPMED and the anticipated process impurities. Conditions: mobile phase, 16% ACN, 84% 70 mM TEA adjusted to pH 4.5 with concentrated phosphoric acid; column, see Figure 2; flow, 2.0 ml/min.; detection, 205 nm ; quantities (mcg), B,46.3; C,42.5; MPMED,38.2; D,37.6; E,53.4; F,41.3; G,32.9.

mobile phase was used. Baseline resolution for all components in the mixture was achieved.

In pH 4.5 TEAP/ACN media, MPMED and the impurities have UV absorbance maxima at 266 nm; however, the absorptivities are higher at wavelengths lower than 220 nm. To provide better sensitivity for

the compounds of interest, 205 nm was used as the detection wavelength. In addition to UV absorption detection, electrochemical detection was considered. It was found that all of the compounds in shown Figure 1 yielded a response when detector potentials in excess of +1.0 volts were employed. The electroactivity is due to the tertiary amine functional group (9,10). Although the sensitivity of the electrochemical detector for compounds of interest was superior to UV detection at 205 nm, UV detection was used for quantitative analysis. The use of TEA as a mobile phase modifier resulted in irreproducible electrochemical detector performance. TEA oxidation products cause high background currents (11).

Method Validation for Quantitative Analysis

The performance of the chromatographic system was evaluated to determine whether or not external standard quantitation could be used to assess the purity of chemical samples of MPMED relative to the MPMED standard. A single standard was employed since the anticipated sample concentration range was narrow (1.0 +/- 0.01 mg/ml). An important factor for quantitation with a single external standard is that the response factor (peak area/conc. or height/conc.)

Table 1. Regression Data for MPMED

	range(mg/ml)	intercept	slope	corr. coeff.
pk. ht.	0.62 - 1.25	0.27	0.63	0.997
areas	0.62 - 1.25	0.04	1.87	0.999

does not change in the anticipated concentration range. Regression data for MPMED peak area and peak height responses are shown in Table 1.

The data indicate that the peak area response is linear while the peak height response is not. Calculation of the response factors over the evaluated range revealed that the peak height response factor decreased 20% while the peak area response response factor changed less than 2%. Quantitative results were based on peak area measurements. A possible explanation for the non-linear peak height response is column overloading. Although retention changes in the evaluated range were not observed, the quantities injected (15-30 mcg) were sufficient to cause slight changes in plate count that result in decreases in the peak-height response factor. The relatively high concentration range was utilized to enhance detection of trace level impurities.

The evaluated chromatographic conditions were used to generate percent purity values for three MPMED lots. Percent purity values are the experimental mg/ml values for the MPMED samples normalized to the actual MPMED levels. The experimental values are based on the response factor of the MPMED standard. Mean percent purity values for the three lots were 98.2%, 97.6%, and 98.3%. The mean values are the average for five determinations. Each determination is based on a separate sample weighing. Relative standard deviation values for the percent purity determinations ranged from 0.69 to 0.96. The percent purity data indicate that the purity of the lots is similar to the purity of the MPMED reference standard.

Qualitative Analysis

An important aspect of initial chemical analysis studies for MPMED is the detection and identification of synthetic process impurities. Initial qualitative studies focused on determining whether or not the anticipated process impurities shown in Figure 1 were present at detectable levels. Figure 4A shows a chromatogram resulting from injection of 25 micrograms of an MPMED chemical sample using UV absorption detection. Impurities with the same

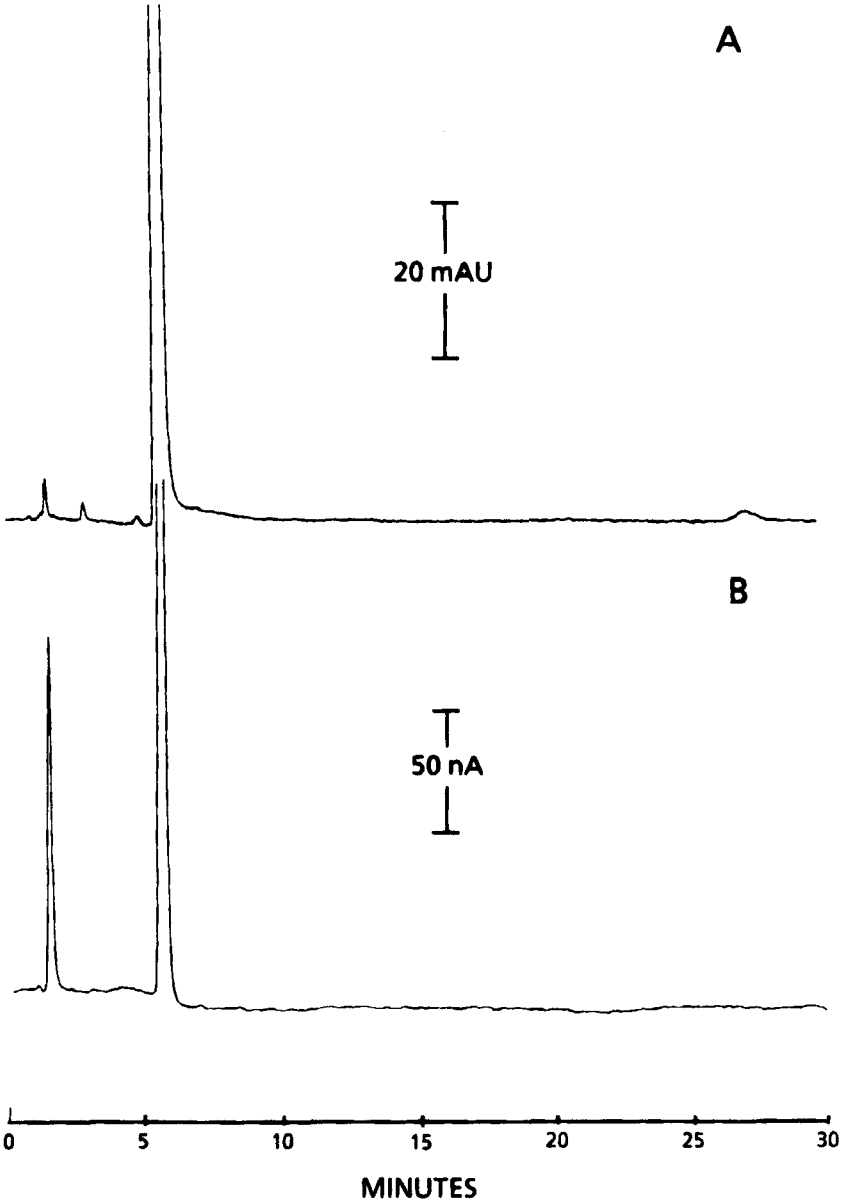

Figure 4. MPMED chemical lot chromatograms. Conditions: see Figure 3; quantity, 25 mcg. detection, A) 205 nM, B) electrochemical detection, +1.0 volt.

retention as the anticipated process impurities were not detected. Several unknown impurities were detected; however, with the exception of the impurity at twenty-six minutes, the area responses of all detected impurities were less than 0.1% when normalized to the MPMED peak area.

Because the anticipated process impurities were not detected with UV absorption detection, the analysis was repeated using electrochemical detection. The chromatogram recorded using electrochemical detection for the same MPMED lot used for Figure 4A is shown in Figure 4B. Qualitative information provided by electrochemical detection was consistent with information provided by UV absorption detection and confirmed that the anticipated process impurities were absent or present at trace levels (< 0.1% of the sample).

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