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Note

Comparison of refractive index, low-wavelength UV and UV visualisation detection methods for the high-performance liquid chromatographic determination of hexamethonium bromide in an injection formulation

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N,N-Hexamethylenebis(trimethylammonium)dibromide, (hexamethonium bromide) a quaternary ammonium ganglion-blocking agent with actions similar to those of pempidine tartrate, is used in the treatment of hypertension and is administered by either subcutaneous or intramuscular injection[1].

The British Pharmaceutical Codex[2] contains a monograph for hexamethonium bromide in which a non-specific titration method is described to determine the purity of the compound. No chromatographic assay procedure appears to have been published. A suitable method is required for the evaluation of hospital manufactured 2.5% (w/v) injection solutions of hexamethonium bromide and, as quaternary ammonium compounds have been assayed previously in this laboratory using ion-pair reversed-phase high-performance liquid chromatography (HPLC)[3,4], this method was chosen for the estimation of hexamethonium bromide. The compound does not contain any UV light absorbing groups and so the classical choice of detection would be differential refractive index (RI) detection. However, papers published in recent years suggest that low-wavelength UV[5] and UV visualisation detection[6] should be considered. In this communication HPLC assays for hexamethonium bromide are presented and the use of the three detection methods evaluated.

EXPERIMENTAL

Instrumentation and reagents

The HPLC system used consisted of an Applied Chromatography Systems 750/03 pump, a Rheodyne 7125 injector valve fitted with a 20-μl sample loop, a Pye-Unicam PU 4020 variable-wavelength absorbance detector operated at 262 and 210 nm, an Applied Chromatography Systems 750/13 differential refractometer, a Perkin Elmer Model 56 dual pen recorder (chart speed, 5 mm/min) with an operating voltage of 10 mV and a Perkin Elmer Sigma 15 integrator. The assays were carried out using a Waters 300 × 4.6 mm I.D. μBondapak C_{18} column. The mobile phases used were: solvent A, methanol–water (50:50) containing 0.005 *M* 1-heptanesulphonic acid sodium salt, pH adjusted to 3.5 with glacial acetic acid; solvent B,

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methanol–water (25:75) containing 0.005 *M* *p*-toluenesulphonic acid sodium salt, pH adjusted to 3.5 with glacial acetic acid. A flow-rate of 2.0 ml/min was found to give acceptable retention times for both solvents.

All solvents and reagents were chromatographic grade (Fisons, Loughborough, U.K.), except toluenesulphonic acid sodium salt (BDH, Eastleigh, U.K.). In all cases pure eluent was used to fill the reference cell of the RI detector.

UV scans of hexamethonium bromide and potassium bromide were carried out using a Perkin Elmer Model 200 UV spectrophotometer.

Methods

A 2.5% (w/v) solution of hexamethonium bromide (Sigma, U.K.) in water was autoclaved at 115°C for 30 min. Samples were then diluted to 0.5% (w/v) before introduction into the injector, using the loop filling technique, with a 100 μl syringe. Standard solutions for the calibration curves were prepared by dissolving a suitable amount of hexamethonium bromide in water.

The RI detector did not have a suitable output for use with the integrator so peak height measurement was used to quantify the results, but both peak height and area were used to calculate the results from the UV detector.

RESULTS AND DISCUSSION

Quaternary ammonium compounds have been assayed successfully in this laboratory by reversed-phase HPLC using an alkylsulphonic acid (usually heptanesulphonic acid) as an ion-pairing agent[3,4]. As a result, the initial assay development work on hexamethonium bromide was carried out using a RI detector and 0.005 *M* heptanesulphonic acid sodium salt in the solvent and varying the methanol–water content to obtain a suitable retention time and to try to detect breakdown products which may be present in the autoclaved injection solution. No breakdown products were detected and the most suitable solvent mixture was found to be solvent A, which gave a retention time for hexamethonium of 4.7 min (Fig. 1a). Linear response was found, using peak height measurement, over the range 80–110 μg injected on column. The minimum detectable quantity of hexamethonium bromide was found to be 2.5 μg.

The UV spectrum of a 0.5% (w/v) solution of hexamethonium bromide shows an E_{max} at around 210 nm. As a result low-wavelength HPLC detection was investigated using solvent A with the UV detector set at 210 nm and 0.04 A.U.F.S. The UV detector was operated in series with a RI detector. Fig. 1b shows that no peak was detected for hexamethonium at 210 nm and so this is not a suitable method of detection for this compound. The UV spectrum of potassium bromide indicates that the low-wavelength UV absorbance exhibited by hexamethonium bromide is due to the bromide in the compound.

UV visualisation detection of hexamethonium bromide was investigated using solvent B containing toluenesulphonic acid as the UV absorbing counter-ion. The UV detector was set at 262 nm, the E_{max} for toluene sulphonic acid, and 0.16 A.U.F.S. The UV detector was operated in series with the RI detector operated at range × 1. Retention times for hexamethonium bromide for RI and UV visualisation detection were 4.0 min (Fig. 2a) and 3.7 min (Fig. 2b) respectively. Linear calibration

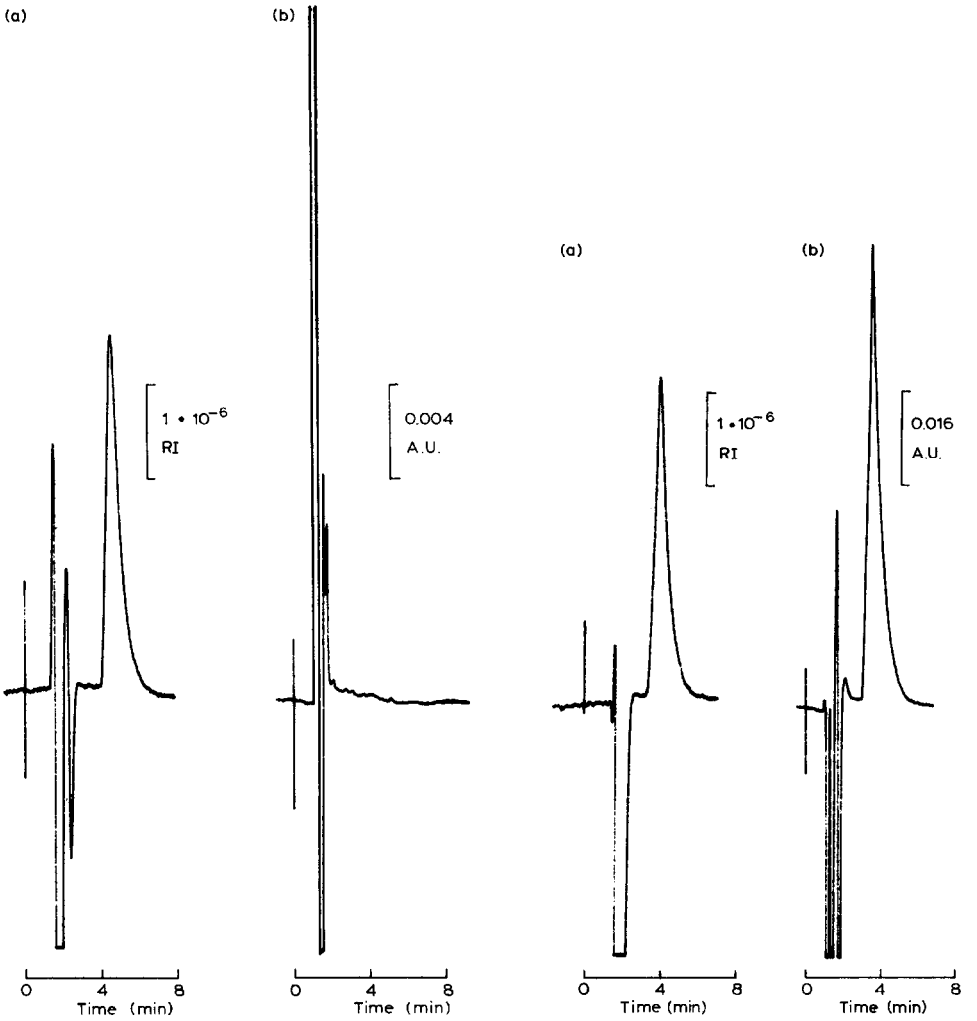

Fig. 1. Chromatograms of 0.5% (w/v) hexamethonium bromide using (a) RI detection (range × 1) and (b) UV detection 210 nm (0.04 A.U.F.S.). Column: μBondapak C_{18}. Eluent: methanol–water (50:50) containing 0.005 *M* heptanesulphonic acid sodium salt, pH 3.5. Flow-rate: 2 ml/min.

Fig. 2. Chromatograms of 0.5% (w/v) hexamethonium bromide using (a) RI detection (range × 1) and (b) UV visualisation detection 262 nm (0.16 A.U.F.S.). Column: μBondapak C_{18}. Eluent: methanol–water (25:75) containing 0.005 *M* toluenesulphonic acid sodium salt, pH 3.5. Flow-rate: 2 ml/min.

curves were obtained for the UV visualisation detection method, using both peak height and peak area measurements, and for the RI detection method using peak height measurement over the range 80–110 μg on column. The minimum detectable quantity of hexamethonium bromide using UV visualisation detection was 0.5 μg (0.04 A.U.F.S.) and for RI detection 2.5 μg (range × 1).

Using a 0.1% (w/v) solution of toluenesulphonic acid sodium salt the system peak for solvent B was found to elute at the solvent front.

TABLE I

ASSAY RESULTS FOR 2.5% (w/v) HEXAMETHONIUM BROMIDE INJECTION USING REFRACTIVE INDEX AND UV VISUALISATION DETECTION METHODS

Detection method	*Quantification method*	*Label claim found (%)*
Refractive index	Peak height	102.75
UV visualisation	Peak height	103.50
UV visualisation	Peak area	103.00

The loop injection valve gave good peak height and area reproducibility for both detection methods, with a relative standard deviation of ± 1.1%.

Stabilisation time for the RI and UV detectors using both solvents A and B was approximately 60 min.

Table I shows the assay results obtained for a production batch of 2.5% (w/v) hexamethonium bromide, 5 ml injection, using solvent B and both RI and UV visualisation detection. The results all fall within the limits: mean ± 0.5% showing that the three quantification methods give very comparable results. Assay limits for the injection were set at 95–105% label claim.

CONCLUSIONS

Of the three HPLC detection methods evaluated for the assay of hexamethonium bromide, RI and UV visualisation give acceptable results, but low-wavelength UV detection, at 210 nm, is unable to detect hexamethonium. The UV visualisation detection method is more sensitive than the use of a differential refractometer and eliminates the need for this latter type of detector for the assay of this non-UV light absorbing compound. From the results obtained it is concluded that the HPLC procedure described is a suitable method for determination of hexamethonium bromide in injection solutions.

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