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Short communication

Validation of a high-performance liquid chromatography method for the determination of pancuronium in Pavulon injections

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Abstract

A new high-performance liquid chromatography (HPLC) method was developed for the quality control of pancuronium bromide and its degradation products. The HPLC method used a 5-μm Supelcogel ODP-50 (150×4 cm) column with acetonitrile–CH_3OH–water–F_3CCOOH (20.5:74.9:0.1, v/v) as the mobile phase (pH value 2.0 adjusted with trifluoroacetic acid) at a flow-rate 0.8 ml/min and UV detection at 210 nm. The Beer's law plots were found to be linear over the concentration range 0.4–1.2 mg/ml of pancuronium bromide and 0.04–0.08 mg/ml of desacetyl degradation products (R^2=0.9995). The RSD of the peak areas was 1.09% and the recovery was 102.43%. The RSD value shows good precision, acceptable accuracy and reproducibility of the new method for the determination of pancuronium bromide in presence of its desacetyl degradation products. The method is rapid and sensitive enough to be used for Pavulon injection analysis.

Keywords: Pharmaceutical analysis; Validation; Pancuronium

1. Introduction

Pancuronium {1,1′-[(3,17-bis(acetyloxy)androstane-2,16-diyl] bis(1-methylpiperidinium) dibromide} is the first synthesized ammonium steroid. In therapy it is used in injection form.

Analytical methods described for the quantitative analysis of pancuronium bromide in injections involved spectrophotometry [1,2], spectrofluorometry [3,4] HPLC [5–8], GC–MS [9,10], LC–MS [11–17] and MS [18] in blood, serum and urine. Consequently the aim of this study was to propose an HPLC method for the determination of pancuronium bromide in injections and to investigate the precision, accuracy and reproducibility of working conditions for HPLC determination.

2. Experimental

2.1. Equipment

Pancuronium bromide was analysed on a GBC LC apparatus and with a 5-μm Supelcogel ODP-50 (150×4 cm) column (Supelco, Bellefonte, PA, USA). The injection volume was 20 μl (injector Rheodyne 7125, USA) elution was performed at a flow-rate of 0.8 ml/min and the column was maintained at ambient temperature. A GBC LC 1210 UV–Vis detector (Australia) was used for monitoring at 210

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nm with 0.05 AUFS. Results were evaluated using software Winchrom chromatography data system v.1.3, GBC Scientific Equiment on personal computer.

2.2. Materials and chemicals

Standards of pancuronium bromide and the Pavulon injections were obtained from Organon Teknika (Boxtel, The Netherlands). Methanol and trifluoroacetic acid (TFA) were supplied by Merck (Darmstadt, Germany) and acetonitrile by Fluka (Buchs, Switzerland). All the solvents used for the preparations of the mobile phase were of HPLC grade and mixtures were filtered and degassed before use. Natrium chloride (Sigma, Buchs, Switzerland), was used for adjusting isotonic solutions and natrium acetate (Chemica, Zagreb, Croatia) and acetic acid were used as buffer system.

2.3. Solutions

Solution P consisted of an acetate buffer solution prepared by mixing 800 mg of sodium acetate trihydrate and 0.26 ml glacial acetic acid and 200 mg sodium chloride with water to obtain 50 ml of solution having a pH 4.0±0.1 in purified water.

Solution R1 consisted of solution P–acetonitrile (75:25, v/v).

For a stock standard solution of pancuronium bromide, about 50 mg of pancuronium bromide reference material were precisely weighed and dissolved in solution R1 and diluted to 25 ml with the same solvent. The concentration of solution was 2.0 mg/ml pancuronium bromide.

For a stock standard solution of 3- and 17-desacetylpancuronium bromide, about 10 mg of 3-desacetylpancuronium bromide and 10 mg 17-desacetylpancuronium bromide reference materials were precisely weighed and dissolved in 6 ml solution P and diluted to 10 ml with the same solvent. The concentrations of the solution were 1.0 mg/ml for both 3-desacetyl- and 17-desacetylpancuronium bromide (dacuronium bromide).

2.4. Preparation of calibration curve

Volumes of 2.0, 3.0, 3.5, 4.0, 4.5, 5.0 and 6.0 ml of the stock standard solution of pancuronium bromide were accurately transferred into seven 10-ml volumetric flasks and diluted to volume with the solution R1. The concentrations of the solutions were 0.4–1.2 mg/ml.

A calibration curve for the determination of the content of 3-desacetyl- and 17-desacetylpancuronium bromide was prepared with volumes of 0.40, 0.50, 0.55, 0.6, 0.65, 0.70 and 0.80 ml of stock standard solution, adding 5.60, 5.50, 5.45, 5.40, 5.35, 5.30 and 5.20 ml acetate buffer solution (pH 4), respectively, and diluted to 10.0 ml with mobile phase; the concentration range was 0.04–0.08 mg/ml for each hydrolysis product.

2.5. Working standard solutions

The working standard solution for pancuronium bromide was prepared by dilution of 4 ml stock standard solution to 10 ml with solution R1. Then solutions were prepared. The concentrations of solutions were 0.8 mg/ml. The working standard solution for the investigation of the desacetyl degradation products was prepared by adding 5.40 ml acetate buffer solution (pH 4) to 0.6 ml stock standard solution of impurities and then diluting to 10.0 ml with the mobile phase (0.06 mg/ml).

2.6. Sample solutions

The content of one Pavulon 4-mg (2-ml) ampoule was diluted to 5.0 ml in a volumetric flask with solution R1. The concentration of pancuronium bromide was 0.8 mg/ml. The sample solution for investigation the presence and quantification of desacetyl degradation products was prepared by diluting 3 ml of the injection solution to 5.0 ml with mobile phase; The concentration of pancuronium bromide was 1.2 mg/ml. In both determinations seven prepared samples were used. The solution was filtered through a 0.2-μm Millipore filter.

2.7. Chromatographic procedure

Three injections (20 μl) of each of these solutions were made into the chromatographic system. The areas of the peaks were measured. For the calibration curve the average peak area for each dilution was plotted against the quantity of pancuronium bromide,

3-desacetyl- and 17-desacetylpancuronium bromide in the solution.

3. Results and discussion

The working conditions for the HPLC method was established with pancuronium bromide bulk drug and then applied on the liquid dosage forms. Fig. 1 shows a chromatogram obtained after injection 20 μl of working standard solution. Under the experimental conditions investigated, the retention time for pancuronium bromide was 6.3 min. The retention time and capacity factor $k'=2.15$ indicated optimal experimental conditions.

After the chromatographic study, the quantitative application of the method was investigated. The method was tested for specificity, linearity, precision, selectivity and reproducibility. The specificity of the method was investigated by observing potential interferences between pancuronium bromide and degradation products 3-desacetyl- and 17-desacetylpancuronium bromide. No interfering peaks were present in the chromatograms. The linearity of the relationship between peak area and concentration was determined by analysing seven standard solutions over the range 0.4–1.2 mg/ml for pancuronium bromide and 0.04–0.08 mg/ml desacetyl degradation products. Each of these solutions (10 μl) was injected three times into the chromatographic system. The regression equations were $y = 5764755.24x + 18568.38$ ($t_a = 0.4351$, $t_{0.05} = 2.0831$), $y = 9977576.19x + 14981.85$ ($t_a = 1.0874$, $t_{0.05} = 2.093$) and $y = 10093320.62x - 28855.91$ ($t_a = -0.9513$, $t_{0.05} = 2.093$) for pancuronium bromide, 3-desacetyl- and 17-desacetylpancuronium bromide, respectively (where t_a is calculated deviation value for intercept and $t_{0.05}$ is tabular deviation value for 0.05 possibility).

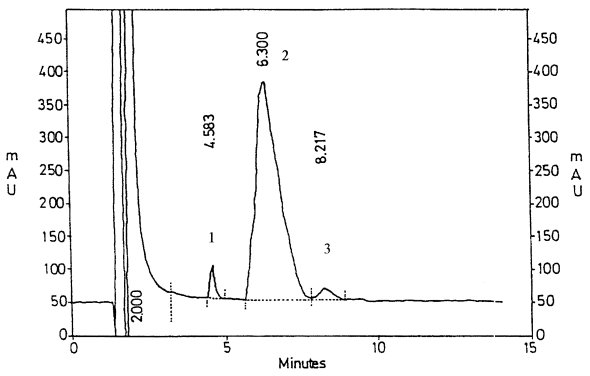

Fig. 1. Chromatogram of pancuronium bromide (2) in the presence of 3-desacetyl- (1) and 17-desacetyl- (3) degradation products on optimal conditions. Eluent: CH_3CN–CH_3OH–water–TFA (20:5:74.9:0.1, v/v), pH 2; flow-rate, 0.8 ml/min.

Coefficients of correlation of calibration curves were greater than $R^2=0.995$. The linearity was confirmed by calculating the relative standard deviation of experimental to theoretical values (0.81, 0.94 and 1.06% for pancuronium bromide, 3-desacetyl- and 17-desacetylpancuronium bromide, respectively) and the detector response factors (RSDs were 1.36, 2.31 and 4.76% for pancuronium bromide, 3-desacetyl- and 17-desacetylpancuronium bromide, respectively).

The limits of detection (LODs) were 6.25, 1.25 and 2.50 μg/ml for pancuronium bromide, 3-desacetyl- and 17-desacetylpancuronium bromide, respectively, which points to a good sensitivity of the method. LODs were measured as the lowest amount of analyte that may be detected to produce a response which is significantly different from that of a blank. The limits of quantification (LOQs) of 20.63, 4.12 and 8.25 μg/ml give a good precision and acceptable accuracy (RSD<3%).

Analysing seven solutions of all three substances following concentrations of pancuronium bromide, 3-desacetyl- and 17-desacetylpancuronium bromide, 0.8, 0.06 and 0.06 mg/ml, respectively, assessed the precision of the chromatographic procedure. The results are presented in Table 1. The good RSD values show satisfactory repeatability of the system.

Accuracy for pancuronium bromide was also investigated. The range of recovery values obtained was 98.17–101.90% (RSD=1.23%). The ranges of recovery values for 3-desacetyl- and 17-desacetylpancuronium bromide for investigating accuracy were: 98.60–102.46% (RSD=1.47%) and 97.50–101.82% (RSD=1.31%), respectively.

The precision of the chromatographic procedure was assessed by analyzing seven solutions containing known quantities of the investigated compounds (0.8 mg/ml for pancuronium bromide and 0.06 mg/ml for 3-desacetyl- and 17-desacetylpancuronium bromide). The RSDs show the satisfactory repeatability of the system (Table 1).

Table 1
Precision of the assay expressed as percent RSD of seven samples for three concentrations

Sample (standard solution) ($n=7$)	Conc. (mg/ml)	Found (mg/ml)	SD	RSD (%)	Recovery (%)
Pancuronium bromide	0.800	0.802	0.0087	1.09	98.53–101.43
3-Desacetylpancuronium bromide	0.060	0.060	0.0016	2.68	95.66–103.00
17-Desacetylpancuronium bromide	0.060	0.059	0.0044	7.30	91.64–108.53

Table 2
Statistical analysis of results in the determination of pancuronium bromide, 3-desacetyl- bromide and 17-desacetylpancuronium bromide in Pavulon, 4 mg = 2-ml injections

Sample ($n=7$)	Found (mg/ampoule)	SD	RSD (%)	Recovery (%)
Pancuronium bromide	4.121	0.0337	0.82	101.84–104.31
3-Desacetylpancuronium bromide	0.108	0.0042	3.92	2.58–2.83
17-Desacetylpancuronium bromide	0.023	0.0065	28.43	0.83–1.22

The reproducibility of the method was investigated by analysing 10 Pavulon ampoules. A summary of the results is presented in Table 2. The good recoveries and low RSDs confirm the suitability of the proposed method for the routine determination of pancuronium bromide in presence of the degradation products 3-desacetyl- and 17-desacetylpancuronium bromide.

The proposed HPLC method is an efficient method for the separation and quantitative determination of pancuronium bromide and its degradation products in dosage forms. There was no interference in the products examined which confirms good selectivity of the method. The method is rapid and sufficiently sensitive for routine analysis.

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