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Determination of isoproterenol sulfate on surfaces using high-performance liquid chromatography with electrochemical detection

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Abstract

Trace amounts of isoproterenol (IP) sulfate are determined on surfaces using high-performance liquid chromatography with electrochemical detection (LC–ED). The drug residues are removed from surfaces with cotton swabs using an aqueous buffer which is 0.05 *M* phosphate, 5 m*M* pentanesulfonic acid and 0.1 m*M* EDTA at pH 3.6. The drug substance is chromatographed using a 15 cm × 4.6 mm I.D. Nucleosil C_{18} column and an eluent containing the same buffer and methanol as a modifier which are mixed in a 90:10 ratio. Detection is performed using an amperometric thin-layer cell with a glassy carbon working electrode operated at +0.65 V versus Ag/AgCl which gives a linear response ($r = >0.9999$) for isoproterenol sulfate to at least 42.9 ng/ml. Repeatability of the chromatographic finish was demonstrated by replicately chromatographing IP solutions over several days. Repeatability (R.S.D. values) of the peak areas ranged from ±0.32% to ±3.0%. For additions of 40 to 120 ng of isoproterenol sulfate average recoveries from cotton swabs ranged from 82 to 98%. At the same addition levels, average recoveries ranged from 87% to 95% for stainless steel and glass surfaces. The precision for all recovery data (R.S.D. values) ranged from ±2.1% to 12.8%.

Keywords: Isoproterenol sulfate

1. Introduction

Current U.S. good manufacturing practices (GMP) require that shared areas and equipment used in drug manufacturing and formulation have established cleaning protocols to avoid cross-contamination of drug products [1]. Drug residues are typically sampled after cleaning and drug concentrations are determined to insure compliance with a targeted limit. This laboratory has previously reported the determination of cephalosporins and *o*-phenylenediamine in such applications [2,3].

Here we report a simple, rugged and specific determination of isoproterenol (IP) sulfate at trace levels from surfaces as an analytical method for support of a cleaning protocol. IP is a potent bronchodilator which is formulated as either the hydrochloride or sulfate salt [4]. Due to the extreme activity of IP, a very sensitive assay was

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necessary that could reliably quantitate IP rapidly in order to minimize the down time of manufacturing equipment and shared production areas. Liquid chromatography with electrochemical detection (LC–ED) is a well-established method for determining catecholamines at low levels. Determination of IP and its *O*-methylated metabolite in physiological salt solutions has been reported by this technique [5]. Attempts to improve selectivity and sensitivity of IP from various biological matrices have used LC with coulometric array detection [6]. An alternate approach involved various column-switching schemes [7] or extensive sample pretreatment [8,9].

In the present paper, adequate sensitivity of IP was obtained by direct injection using modest injection volumes and minimal sample pretreatment. Recovery data of the IP sulfate salt from various surfaces and assay performance are described in detail.

2. Experimental

2.1. Equipment

The liquid chromatograph used consisted of a Model PM-80 isocratic pump, a Model LC-4C amperometric detector and a Model CC-5 cell stand with a fixed loop injection valve (Bioanalytical Systems, West Lafayette, IN, USA). The working electrode was a thin-layer glassy carbon electrode and all measurements were made versus a Ag/AgCl reference electrode (Bioanalytical Systems). A Chromjet data-handling system was used with a 10 mV input (Spectra-Physics, San Jose, CA, USA). Chromatographic separations were achieved using a 15 cm × 4.6 mm I.D. Nucleosil C_{18} column (5-μm packing, 120 Å pore size) (Chromatography Sciences, St.-Laurent, Canada). Cotton-tipped applicators were used for sampling (Baxter, McGaw Park, IL, USA). Sample and standard preparations were filtered through 0.20-μm PVDF membranes and the HPLC eluent was filtered through 0.45-μm nylon membranes prior to use (Gelman Sciences, Ann Arbor, MI, USA).

2.2. Reagents

Potassium phosphate monobasic was reagent grade and methanol was HPLC grade (EM Sciences, Gibbstown, NJ, USA). Disodium EDTA and 1-pentanesulfonic acid, sodium salt were reagent grade (Aldrich Chemical, Milwaukee, WI, USA). IP hydrochloride standard was obtained from USP. Bulk IP sulfate was purchased as a bulk commodity drug from commercial sources. The eluent buffer, prepared in deionized water, was 0.05 *M* in potassium phosphate, 5 m*M* in 1-pentanesulfonic acid, sodium and 0.01 m*M* in disodium EDTA. The solution pH was adjusted to 3.6 using small volumes of phosphoric acid. The HPLC eluent was a mixture of 900 ml of the eluent buffer and 100 ml of methanol. The eluent was vacuum-filtered prior to use.

2.3. Standard and sample preparation

A stock solution of IP hydrochloride at 100 μg/ml was prepared in the eluent buffer. This solution was diluted serially in the eluent buffer to a working standard preparation containing approximately 8 ng/ml of IP hydrochloride. For surfaces, 10 ml of the eluent buffer was pipetted into screw cap vials. Areas of 100 cm^2 were sampled by swabbing the surface with the buffer solution using a cotton-tipped applicator. The applicator was frequently immersed in the solution in the vial to remove as much surface residue as possible. Residual buffer on the surface was sorbed onto a second applicator. The applicators were extracted with the solution using mechanical shaking for 20 min. Samples and standards were filtered through 0.20-μm PDVF membranes prior to injection. An extraction blank was prepared by extracting two applicators without sampling and filtered similarly.

2.4. Typical chromatographic conditions

The electrochemical detector was operated at an applied potential of +0.65 V versus Ag/AgCl. A glassy carbon working electrode was used and

the detector range was 5 nA/FS (attenuation 1–4). A 15 cm × 4.6 mm I.D. Nucleosil C_{18} column was used with an eluent of 90% (0.05 *M* phosphate, 5 m*M* 1-pentanesulfonic acid, sodium and 0.1 m*M* Na_2EDTA at pH 3.6)–10% methanol. Flow-rate was 1.0 ml/min producing approximately 1600 p.s.i. backpressure; 50-μl injection volumes were used.

2.5. Assay procedure

The standard preparation was injected and the chromatographic conditions were adjusted as necessary to obtain an IP peak at 6–11 min having ≥25% full scale response. The tailing factor (*T*, USP XXIII) was calculated for IP, where: $T = W_{0.05}/2(f)$; $W_{0.05}$ is the peak width at 5% height and f is the width of the peak from the peak maximum to the leading edge of the peak. A value of $T \leq 1.5$ was judged acceptable for the determination. The standard preparation was injected replicately to obtain an average peak area with relative standard deviation of ±6% or better for at least four consecutive injections. The blank extraction preparation was used to verify that no significant response occurred at the retention time of IP. Each sample preparation was injected and the amount of IP sulfate was determined using the following equation:

$$\text{ng IP sulfate}/100\ \text{cm}^2 = \frac{PA\ (\text{Spl.})}{\text{Avg. } PA\ (\text{Std.})} \times \text{Std. Conc. (ng/ml)} \times \frac{260.30}{247.72} \times 10\ \text{ml}$$

where *PA* (Spl.) is the peak area of the sample and Avg. *PA* (Std.) is the average peak area of the IP hydrochloride standard.

3. Results and discussion

The assay conditions used in this work provided consistently low detector background currents (typically 0.2–0.3 nA). Shown in Fig. 1 is a hydrodynamic voltammogram constructed by chromatographing 1.02 ng of IP hydrochloride

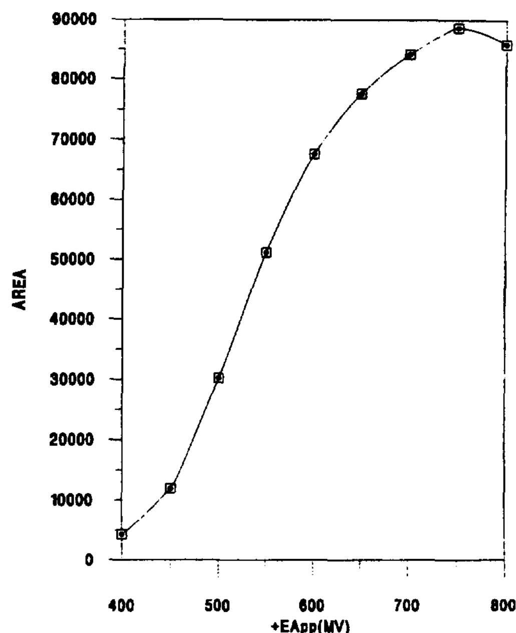

Fig. 1. Hydrodynamic voltammogram for IP.

using applied potentials of +0.40 to +0.80 V versus Ag/AgCl and the chromatographic conditions described. A working electrode potential of +0.65 V gave the most acceptable compromise to providing good sensitivity and specificity while approaching the maximum of the potential versus response curve. Higher potentials provided slightly better absolute response of IP; however, greater detector noise and extraneous peaks extracted from samples were observed. Using the stated chromatographic conditions, a plot of IP sulfate response (y = area, counts) versus concentration (x = ng/ml) was linear over the concentration range of 2.1 to 42.9 ng/ml. A six-point regression line had a correlation coefficient >0.9999, slope = $3.70 \cdot 10^3$ (S.D. = $\pm 0.02 \cdot 10^3$) and a y-intercept of −526 (S.D. = ±341).

Shown in Fig. 2 are typical chromatograms from this work. This figure shows three chromatograms which are a standard preparation, a sample preparation from a stainless steel surface and a sample preparation from a stainless surface in which 80 ng of IP sulfate was added. As the assay procedure is presented, approximately 341 pg (1.6 pmoles) of IP base is injected on-column

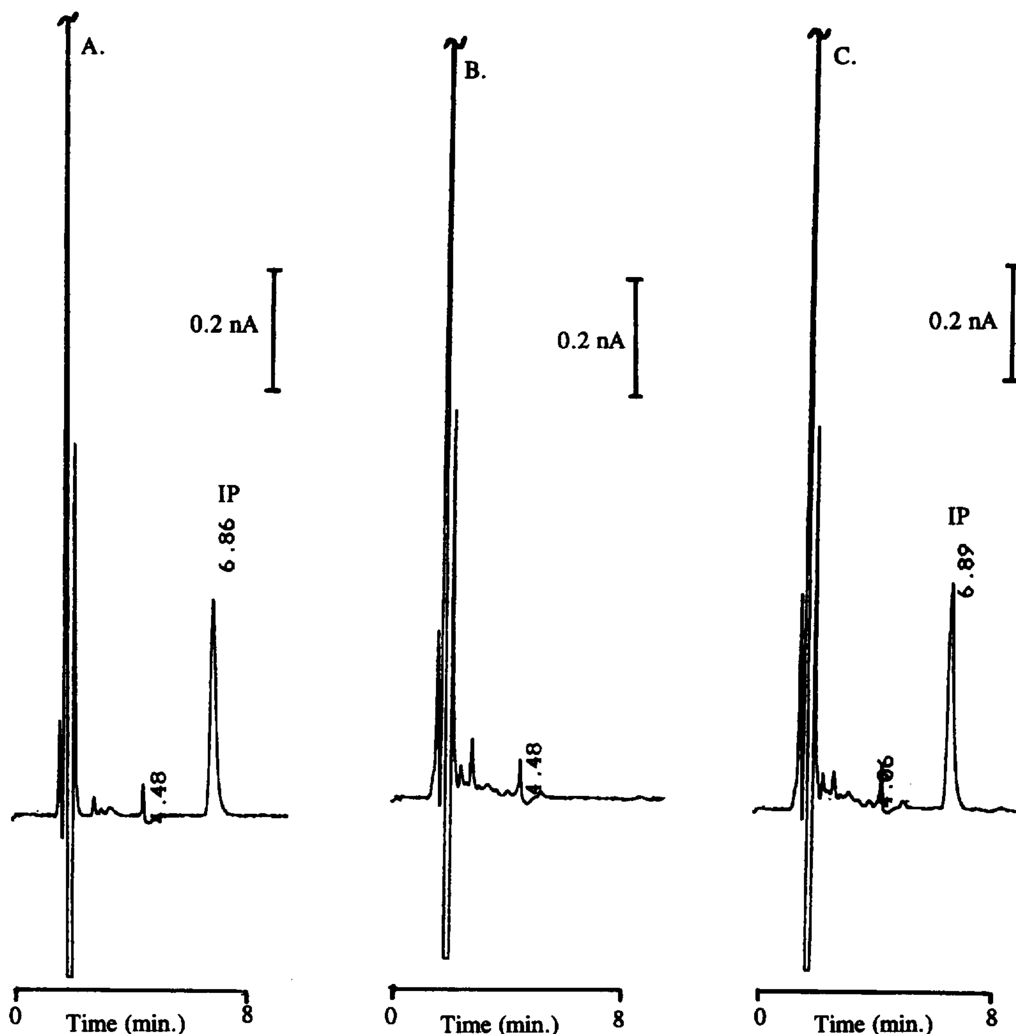

Fig. 2. Typical chromatograms for IP sulfate determination. (A) Standard preparation (8 ng/ml IP hydrochloride); (B) sample preparation, stainless steel surface; (C) sample preparation, stainless steel surface with 80 ng of IP sulfate added over 100 cm^2. Chromatographic conditions are stated in text.

in the standard preparation. This amount of IP was chosen as a working limit on the basis of the activity of the drug. The actual detection and quantitation limits of the method were evaluated by chromatographing standards and spiked sample preparations. Shown in Fig. 3 are chromatograms of a 0.20 ng/ml IP hydrochloride standard preparation, a blank extraction and a sample preparation spiked with 0.10 ng/ml of IP hydrochloride. IP sulfate is detectable at 0.10 ng/ml (4.3 pg or 0.02 pmole IP base on-column, S/N approx. 3) and determinable at 0.20 ng/ml (R.S.D. ±10.5% and $n = 4$). The detection limit observed in this work is slightly lower than cited in the literature (0.1 pmole; [5]). This probably results from a cleaner sample matrix and lower operating potential used here.

In order to evaluate the robustness of the chromatographic separation, IP was chromatographed after intentional variations in eluent organic modifier composition, eluent buffer pH and ionic strength were made. IP retention times

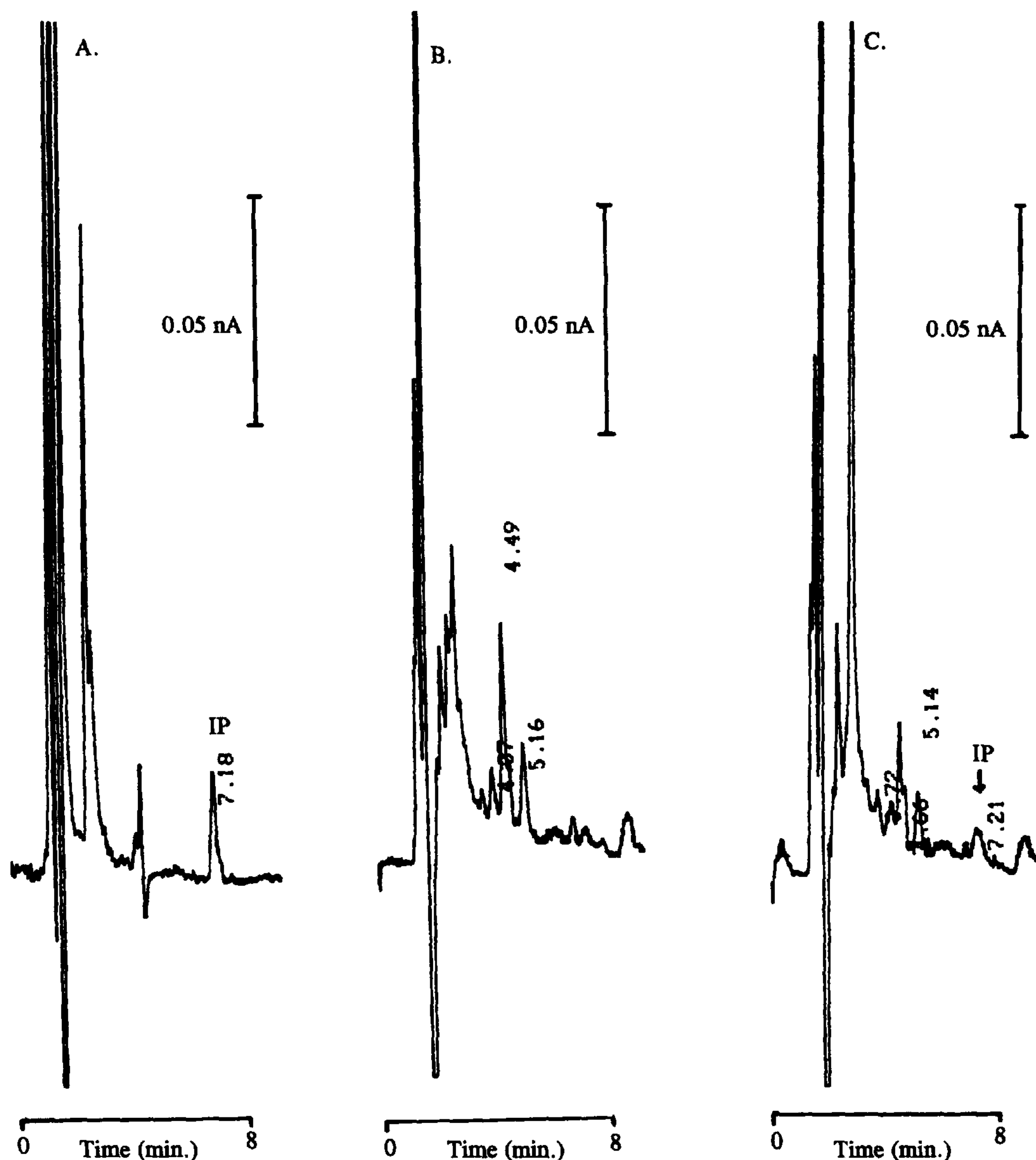

Fig. 3. Chromatograms for IP at high gain. (A) Standard preparation (0.2 ng/ml IP hydrochloride); (B) extraction blank; (C) sample preparation containing 0.1 ng/ml IP hydrochloride. Chromatographic conditions are stated in text (integrator attenuation increased to 0.5).

(t_R) and calculated tailing factors (T) after these variations were made are summarized in Table 1. As shown, very little peak tailing occurred under any conditions tested. The most significant changes occurred in t_R on variation of the methanol content of the eluent. Very reproducible chromatographic performance was obtained throughout this work using different columns and analysts. Nearly identical retention times and T values were obtained for columns packed with different batches of the specified stationary phase.

The repeatability of the method was evaluated by chromatographing freshly prepared solutions

Table 1
Tailing factor (T) and retention time (t_R) for IP sulfate as a function of eluent composition

Buffer (%)	Methanol (%)	Ionic strength of the buffer[a] (%)	Buffer pH	T	t_R
90[b]	10	100	3.6	1.3	8.7
90[b]	10	100	3.6	1.1	8.9
87.5	12.5	100	3.6	1.1	6.7
92.5	7.5	100	3.6	1.1	13.6
90	10	100	3.0	1.2	9.5
90	10	100	4.2	1.2	9.3
90	10	80	3.6	1.2	8.7
90	10	120	3.6	1.1	9.3

[a] Relative to the intended ionic strength defined by the method.
[b] Intended composition of the method's eluent, on separate days.

of IP hydrochloride and IP sulfate. At 8.2 ng/ml, the average IP hydrochloride peak area was $28.7 \cdot 10^3$ counts with an R.S.D. value of ±1.8% (5 injections). At 8.0 ng/ml, IP sulfate gave average peak areas of $22.6 \cdot 10^3$, $24.3 \cdot 10^3$ and $24.0 \cdot 10^3$ counts on three separations days with R.S.D. values of ±3.0%, ±2.1% and ±0.32% (5 injections each solution), respectively. These data illustrate that the method provides acceptable repeatability for the standard and sample preparations. Moreover, the detector response is comparable for the analyte in a sample preparation over several days at the working limit of IP sulfate.

IP slowly degrades in the preparations described, necessitating chromatographing the solutions on preparation. An 8.0 ng/ml sample preparation of IP sulfate was chromatographed periodically over several days at room temperature. The peak areas were 92.6, 59.0 and 47.0% of the initial peak area, respectively, at day 1, 5 and 6. The stock solution of the standard is stable for at least three days when refrigerated. At three days refrigeration (5°C), 98.8% of the initial concentration (100 μg/ml IP hydrochloride) was present.

Several standard addition and recovery experiments were performed using IP sulfate. Prior to developing the method several different lots of applicators were screened by adding 80 ng of IP hydrochloride to the cotton surfaces and determining the recovery after work-up. Average recoveries of 78.5% (R.S.D. ±9.8%, $n = 4$) to 98.9% (R.S.D. ±3.1%, $n = 4$) were obtained and the lot of applicators providing the most quantitative and reproducible recovery was reserved for this work. These data highlight the possibility of adsorption losses of the analyte at the trace levels studied. Additions of 40, 80 and 120 ng of IP sulfate were made from a 2000 ng/ml stock

Table 2
Average recovery (%) data for IP sulfate from cotton swabs, stainless steel and glass

Amount added (ng)	Swab		Stainless steel		Glass	
	Recovery[a]	Range	Recovery[a]	Range	Recovery[a]	Range
40	98 ± 6.8	91–104	90 ± 2.1	88–93	91 ± 8.2	80–98
80	82 ± 12.8	69–97	90 ± 2.4	89–94	95 ± 5.7	91–100
120	96 ± 4.9	89–99	87 ± 2.8	84–99	89 ± 11.9	72–100

[a] $n = 4$.

solution to moist cotton swabs and surfaces of stainless steel and glass. Multiple additions and recoveries ($n = 4$) were performed for each surface and the results are presented in Table 2. As shown, average recoveries of 82% to 98% were obtained from the cotton applicators. For stainless steel and glass the average recoveries were from 87% to 90% and from 89% to 95%, respectively. The precision for all recovery data (R.S.D. values) ranged from ±2.1% to ±12.8%.

4. Conclusion

This work provides a validated procedure for determining IP sulfate on surfaces. The method is specific, rugged and precise. Recoveries of IP sulfate from glass and stainless steel averaged greater than 87% in the ng range.

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