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Short communication

Determination of trace levels of hydrazine in the penultimate intermediate of a novel anti-infective agent[1]

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1. Introduction

Hydrazine is a common reagent used in the synthesis of pharmaceutical bulk drugs and intermediates. Hydrazine is used in the synthesis of SQ-84251 which is the penultimate intermediate in the synthesis of BMS-180680, a new antibiotic being developed at Bristol- Myers Squibb.

The monitoring of trace levels of hydrazine in bulk drugs or intermediates is necessary due to its possible toxicity. Hydrazine has been analyzed using a number of different techniques including flow injection, gas chromatography (GC) and liquid chromatography (LC). Flow injection methods [1–3] involve electrochemical detection of hydrazine on chemically modified electrodes. GC methods involve derivatization of hydrazine with benzaldehydes [4–6], benzoyltrifluoroacetone [7], acetone [8,9], and *p*-chlorobenzaldehyde [10]. LC is the most commonly used method for the analysis of hydrazine. A derivatization of hydrazine is required for the LC analysis. Most commonly used derivatives are formed with cinnamaldehyde [11,12], salicylaldehyde [13–16], 4-hydroxybenzaldehyde [17], and benzaldehyde [18,19]. GC with Fourier-transform IR has also been used for the analysis of hydrazine derivatives [20]. Electrochemical detection with LC has been used for the analysis of hydrazine [21]. All of these methods (GC and LC) are limited by a time-consuming derivatization process. In addition, the selectivity of hydrazine derivatives cannot be guaranteed during the analysis of pharmaceuticals.

There are no reports in the literature of the analysis of hydrazine using ion chromatography with electrochemical detection. In this paper, a direct chromatographic method on a polymeric CS-14 cation column using electrochemical detection with a platinum electrode, is described for the trace determination of hydrazine (25 ng ml^{-1}) in SQ-84251.

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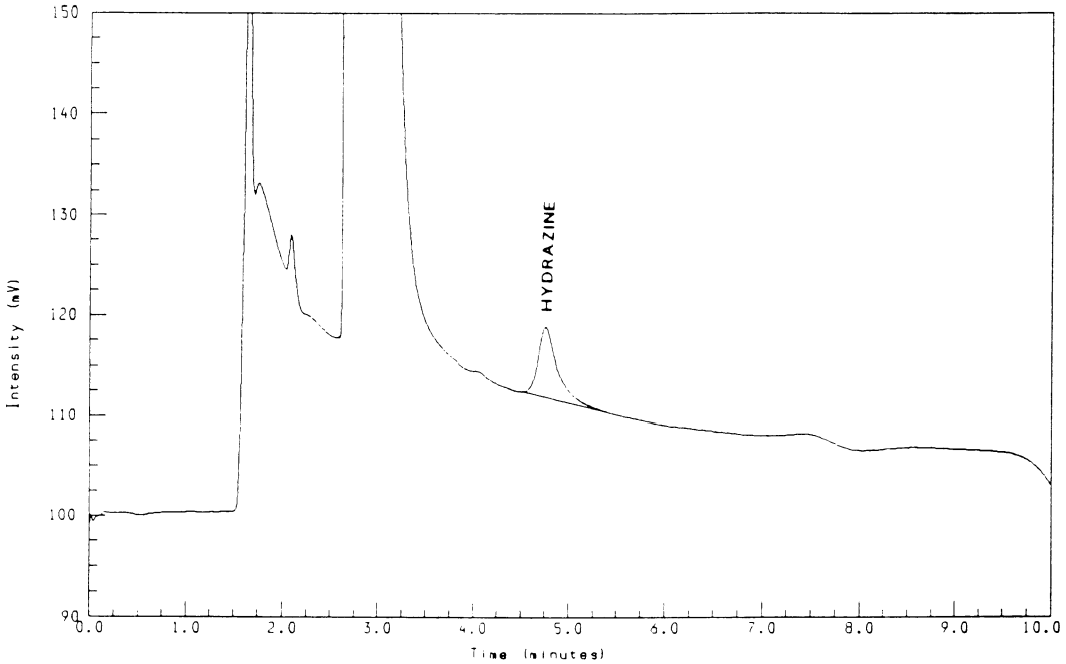

Fig. 1. A batch of SQ-84251 at the working concentration, 500 μg ml^{-1}.

2. Materials and methods

2.1. Reagents

SQ-84251 was obtained from the Chemical Process Technology Group, Bristol-Myers Squibb, New Brunswick, NJ. Perchloric Acid was purchased from Mallinckrodt (Paris, KY). Hydrazine dihydrochloride was purchased from Aldrich (Milwaukee, WI). HPLC grade methanol was obtained from Fisher Scientific (Edison, NJ) and 0.45 μm Nylon-66 filters were obtained from Schleicher and Schuell (Keene, NH).

2.2. Instrumentation

A Dionex DX-500 ion chromatograph composed of a gradient pump module (GP40), eluent degas module (EDM-II), and electrochemical detector (ED-40), all from Dionex (Sunnyvale, CA) was used. The system was connected to a Thermo Separation Products (Fremont, CA) autosampler (model AS3500). Data acquisition was performed with a VG Multichrom data processor (VG Laboratory Systems, Cheshire, WA).

A CS-14 (4×250 mm) column and a CG-14 (4×50 mm) guard column, were purchased from Dionex Corporation (Sunnyvale, CA). The mobile phase was perchloric acid (10 mM) and the sample/standard solvent consisted of methanol and water (50:50 v/v). The flow rate was 1 ml min^{-1}, and the column temperature was ambient.

2.3. Detector conditions

Detection was performed with a Dionex pulsed electrochemical detector using a platinum working electrode and a Ag/AgCl reference electrode. The detector was used in the direct amperometry (DC) mode with the potential set at $+0.8$ V.

3. Results and discussion

A Dionex CS-14 column was chosen for the analysis. The effect of perchloric acid (7–25 mM) concentration on the retention of hydrazine was studied and the best results were obtained with the mobile phase described in the experimental

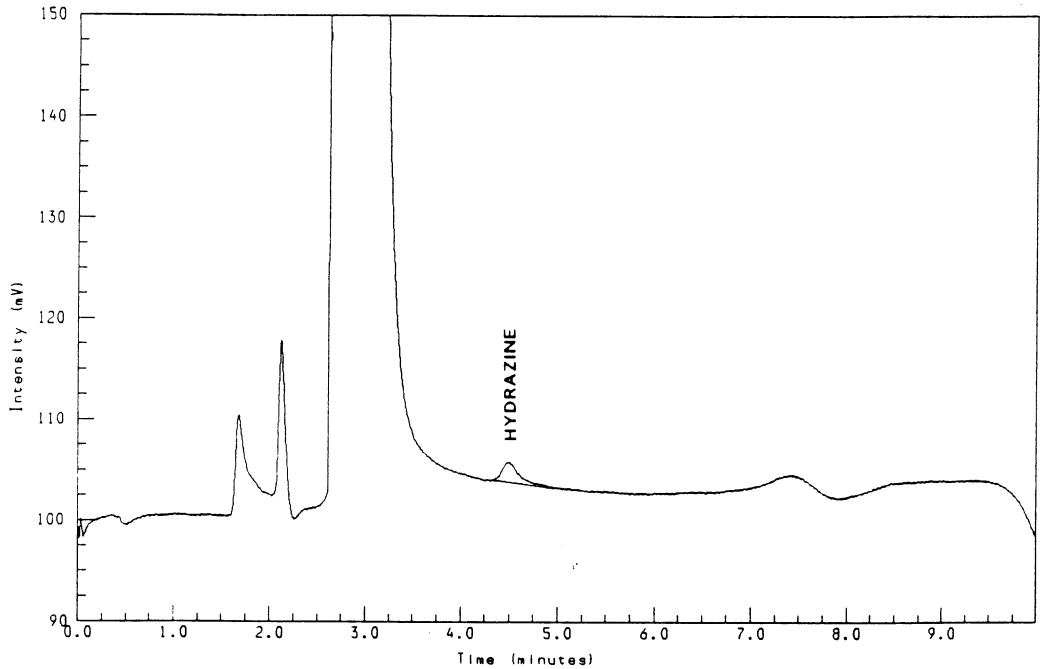

Fig. 2. Typical chromatogram of hydrazine at the limit of detection (25 ng ml^{-1}) at a S/N ratio of five.

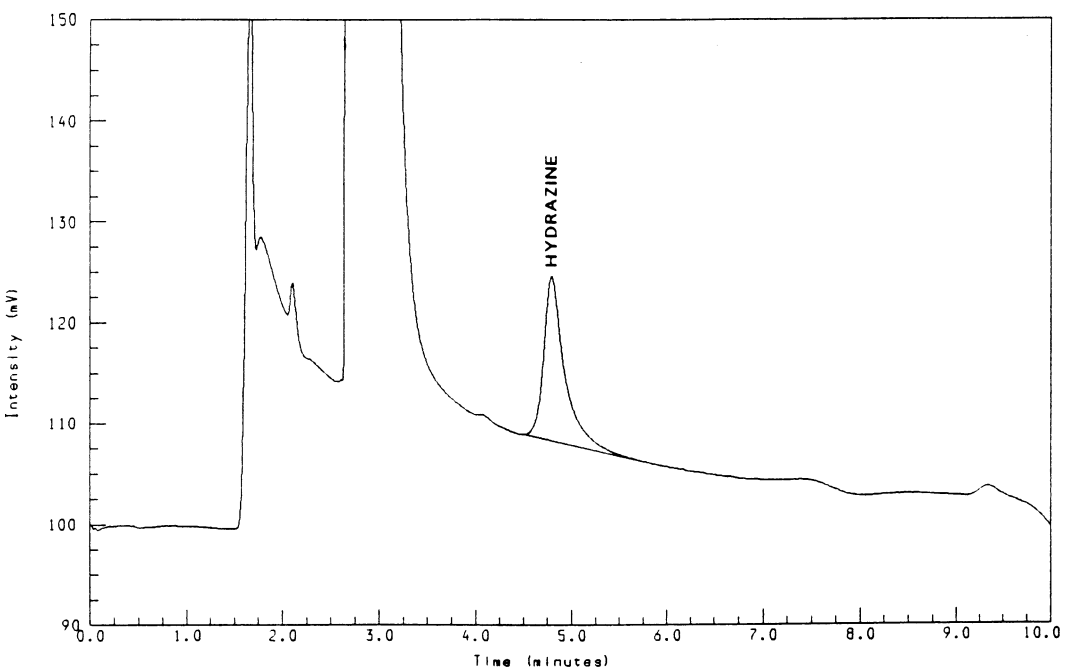

Fig. 3. Typical chromatogram of hydrazine at the miminum quantifiable level (50 ng ml^{-1}) in the presence of SQ-84251(500 μg ml^{-1}).

section. The detection was optimized by varying the oxidation potential from +0.6 to 1.0 V. It was noted that the best detection conditions were obtained at +0.8 V. A major advantage of the method is that SQ- 84251 dissolved in 50:50 v/v methanol and water, can be directly injected into the IC system. Thus, a sample clean-up step normally associated with trace pharmaceutical drug analysis is avoided with saving in time. A chromatogram of a batch of SQ-84251 at the working concentration, 500 μg ml^{-1} is shown in Fig. 1.

Table 1
Precision of the system for SQ-84251 spiked at 0.01% w/w with hydrazine

Injection	Peak area	% hydrazine
1	247257	0.01498
2	246891	0.01496
3	243629	0.01478
4	245239	0.01487
5	243118	0.01475
6	241178	0.01464
7	242904	0.01474
8	238631	0.01450
Mean	243605.9	0.01478
S.D.	2882.6	0.00016
% R.S.D.	1.2	1.1

Table 2
Recovery of hydrazine added to SQ-84251

% added (w/w)	% found (w/w)[a]	% recovered
0	0.00662	—
0.00976	0.01478	90.2
0.01952	0.02742	104.9
0.02928	0.03726	103.8

[a]Based on duplicate injections.

Table 3
Solution stability of hydrazine in sample solvent over 69 h time

Time (h)	% Hydrazine (w/w)[a]
0	0.01498
1.3	0.01450
68.9	0.01446
Mean	0.01465
S.D.	0.00029
R.S.D.	2.0

[a]Based on duplicate injections.

The limit of detection obtained for hydrazine was 25 ng/ml (corresponding to 0.005% w/w of SQ-84251). Fig. 2 shows a typical chromatogram of hydrazine at the limit of detection. The separation conditions are selective and there are no interferences from other impurities originating from the synthetic process of SQ-84251. The minimum quantifiable level of hydrazine was 50 ng ml^{-1} (corresponding to 0.01% w/w of SQ-84251). Fig. 3 shows a typical chromatogram of hydrazine at the minimum quantifiable level in the presence of SQ-84251.

The linearity of the assay was studied from 50 to 200 ng ml^{-1} (corresponding to 0.01–0.04% w/w hydrazine in SQ-84251). The correlation coefficient was >0.99 ($n=5$). The intercept was -1856 and the slope was -24553. A three-point calibration was used for the quantitation of hydrazine. The three standards cover the range of 50–150 ng ml^{-1} (0.01–0.03% w/w).

The precision of the assay was determined by injecting, in replicate ($n=8$), SQ-84251 at 0.5 mg ml^{-1} spiked with 0.01% w/w hydrazine. Table 1 illustrates the precision data.

The accuracy of the method was determined by spiking SQ-84251 with three levels of hydrazine and calculating the recovery. The recovery of hydrazine at levels ranging from 0.01 to 0.03% w/w was 90–105%. Table 2 illustrates the recovery and accuracy data.

Solution stability was determined using a 0.5 mg ml^{-1} solution of SQ-84251 stored at room temperature and assayed after 1.3 and 69 h. There was no noticeable change in hydrazine content in the solution after 69 h and the R.S.D. was 2.0% (Table 3).

In summary, a direct, rapid electrochemical method has been described for the sensitive detection of ultratrace levels of hydrazine (0.005% w/w of SQ-84251) in the penultimate intermediate of a broad-spectrum antibiotic.

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