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Ion chromatographic determination of trace hydroxylamine in waste streams generated by a pharmaceutical reaction process

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Abstract

Hydroxylamine is a key raw material used in a synthetic drug process at Pharmacia. Since hydroxylamine is harmful to microorganisms, concentrations above 5 ppm could interfere with the biological sewage plant performance. This necessitated the development of a sensitive analytical method for detecting low levels of hydroxylamine in the waste streams generated from the pharmaceutical process. The present report describes a cation-exchange chromatographic method coupled with pulsed amperometric detection at a gold electrode for trace analysis of hydroxylamine. This method was evaluated by generating data on the parameters of specificity, precision, linearity, recovery and sensitivity.

Keywords: Validation; Pharmaceutical application; Water analysis; Hydroxylamine

1. Introduction

Hydroxylamine belongs to an important class of reducing agents, which are commonly used as industrial raw materials. It is often used as a raw material for synthesis of pharmaceutical intermediates and final drug substances. However, hydroxylamine is a well-known mutagen, moderately toxic and harmful to microorganisms that could interfere with biological sewage plant performance [1–3]. Because of its bacteriotoxicity, government regulations have called for waste stream levels for hydroxylamine to be under low ppm level to ensure that local marine species are not endangered. This necessitated the development of a sensitive analytical method for detecting low levels (5 ppm) of hydroxylamine in the final waste streams before disposal.

Various methods have been reported for the determination of low levels of hydroxylamine in pharmaceutical analysis. These include gas chromatography with pre-column derivatization [4], gas chromatography with electron-capture detection [5], gas chromatography with mass spectrometric analysis [6], high-performance liquid chromatography (HPLC) with UV detection [7], capillary ion electrophoresis with conductivity and electrochemical detection [8,9], flow injection analysis with amperometric detection ion [10], spectrophotometric determination after derivatization [11,12], and recently ion chromatography (IC) with conductivity and amperometric detection [13,14]. In the past few years, there have been numerous literature regarding application of pulsed amperometric detection techniques combined with anion-exchange chromatography for determination of carbohydrates and other oxidizable species such as amines and sulfur compounds [15–17]. To our knowledge, however, no report concerning pulse amperometric detection of trace levels of hydroxylamine at gold electrode has been made until now.

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The present report describes a direct chromatographic method on a polymeric CS-14 cation-exchange column using pulsed amperometric detection (PAD) towards trace hydroxylamine analysis. The acidic eluent (H_2SO_4) conditions are necessary for protonation of the analytes of interest for chromatographic separation. The detection employs quadruple-potential waveform at gold electrode in high alkaline media, which has been previously developed for carbohydrate applications [18–20]. Hydroxylamines typically do not give persistent amperometric response for a constant (d.c.) applied potential at a platinum (Pt) electrode. However, the hydroxylamine peak area response change (reduction) is minimal overtime with the application of quadruple-potential waveform at gold electrode, which is a vast improvement over the amperometric (d.c.) mode of detection.

The method described in this paper is selective for hydroxylamine and other *N*-alkylhydroxylamines in an ethanol–water matrix. It has been evaluated by generating data on the parameters of specificity, precision, linearity, accuracy/recovery and sensitivity. The multitude of samples that can be analyzed are either filtrates (mother liquor) from isolation of drug substance, filtrates obtained after the first ethanol wash, or filtrates obtained after the second or final water wash or similarly generated in laboratory or plant scale (untreated waste streams).

2. Experimental

2.1. Chemicals and reagents

All chemicals were of analytical reagent grade and all reagents, eluents and standard solutions were prepared using degassed Milli-Q water. The high-purity, deionized water with specific resistance of 18.2 MΩ cm was obtained with a Milli-Q system (Millipore, Bedford, MA, USA) and then filtered through a 0.22-μm Millipak filter.

Hydroxylamine hydrochloride (99.999%) used in stock standard preparation was purchased from Aldrich (Milwaukee, WI, USA). *N*-alkylhydroxylamines (*N*-methylhydroxylamine and *N*,*N*-dimethylhydroxylamine) were obtained from Aldrich for the preparation of marker standards. Concentrated sulfuric acid (98%) was obtained from Fisher Scientific (Edison, NJ, USA) for the preparation of eluent. High-purity-grade NaOH (50%, w/w) purchased from Mallinckrodt (Paris, KY, USA) was used for the preparation of post-column reagent.

2.2. Instrumentation

All experiments were carried out with a DX-500 ion chromatograph (Dionex, Sunnyvale, CA, USA). The system consisted of a quaternary gradient pump (GP40) with automated membrane eluent degassing capability, a chromatographic oven (LC20), an electrochemical detector (ED40), a pneumatic controller with reagent reservoir for post-column eluent delivery (PC10), and an autosampler (AS40). A personal computer equipped with Dionex PeakNet chromatography software (5.2) was used for instrument control. Data collection and data processing were performed with the use of a Perkin-Elmer data acquisition system (Turbochrom 6.1.1).

2.3. Chromatographic conditions

For cation separations (protonated hydroxylamines), an IonPac CS14 (250×4 mm) analytical column and IonPac CG14 (50×4 mm) guard column were purchased from Dionex. The eluent was H_2SO_4 (11 m*M*) and the diluent for sample/standard was Milli-Q water. The column temperature was ambient. Unless otherwise stated, eluent flow-rate was 1.0 ml/min (isocratic). The injection volume for both standard and sample was 50 μl. The total analysis time was 10 min.

A Dionex ED 40 electrochemical detector in the electrochemical integrated amperometric detection mode was used. The ED 40 standard amperometry cell purchased from Dionex was a miniature flow-through cell consisted of a titanium cell body (counter electrode), a combination pH-Ag/AgCl reference electrode and a standard working electrode (gold). The gold electrode was polished once with 1-μm alumina slurry for 1 min and rinsed well with Milli-Q water to eliminate any adsorbed alumina particles. The detector output range was set at 1 μC. The post-column base (300 m*M* NaOH) was added at the rate of approximately 1.2 ml/min by maintaining pressure at solvent delivery system (PC 10) at

approximately 30 p.s.i. (1 p.s.i.=6894.76 Pa). A 375 μl mixing coil was adequate in delivering pH>10 post-column eluent to detector cell. The system is typically equilibrated for 1 h to obtain a stable baseline.

2.4. Sample/standard preparation

The standard stock solution of 100 μg/ml hydroxylamine (NH_2OH) was prepared by weighing 21.0 mg of hydroxylamine hydrochloride [NH_2OHHCl] into a 100 ml volumetric flask. The stock standard was dissolved and diluted to volume with Milli-Q water. All other system suitability standards and other related working standards were prepared by appropriate dilutions of the stock standard using Milli-Q water as the diluent. All final water washes (samples) were prepared at 100 mg/ml concentrations by weighing accurately 1 ml of sample into 10 ml volumetric flask and diluting it to the final volume with Milli-Q water. Appropriate dilutions were made for concentrated samples to obtain comparable peak heights of NH_2OH. The pH values for treated waste streams were typically less than 7. Therefore adjustments for pH during sample preparation were not required in the presence of acidic eluent for chromatographic separation.

3. Results and discussion

3.1. Choice of separation system

A Dionex IonPac CS-14 column was chosen primarily for the hydroxylamine separation. These columns are designed specifically for the separation of alkyl- and alkanolamines from the Group I and Group II cations using sulfuric acid or methanesulfonic acid as acidic eluents. Due to free availability in high pure form, sulfuric acid was chosen as the eluent. The effect of H_2SO_4 (7.5–15 m*M*) on the retention of hydroxylamine was studied and the best results were obtained with the isocratic eluent described in the experimental section. Hydroxylamines do not absorb in the useful UV region significantly. The absorption maximum is in the more noise prone 190–215 nm UV regions, which does not facilitate selective detection. In a typical analytical situation, if the sample contained a significant amount of sodium ions, one would observe a co-elution of both hydroxylamine and sodium ions; hence conductivity detection was not considered for this study due to lack of selectivity. Hydroxylamine has been reported to be electroactive [21], which raises the possibility of using IC with amperometric detection for the selective detection of hydroxylamines because of electroinactive nature of sodium ions. We have found that hydroxylamines can be oxidized with the application of a single positive potential (0.7 V) at a platinum electrode using sulfuric acid as the eluent. If only a single potential is applied to the electrode, oxidation products gradually poison the electrode surface. This electrode poisoning causes the loss of analyte signal, which is indicative of the reduction of peak area response over time. In general, the decrease in peak area response with time due to electrode fouling is a common problem associated with d.c. amperometry, which can be overcome by switching to pulsed amperometry. The data obtained with our preliminary work has indicated that hydroxylamine (0.5 μg/ml) peak area response decreases over a 15 h period to about 28% with the application of constant potential (d.c.) at a Pt electrode. The relative standard deviations (RSDs) for six replicate injections within the above time frame were almost 10%. This led us to investigate a pulse amperometric technique, which supposedly would prevent the continuous signal loss when the electrode surface is cleaned by a series of potentials that are applied for fixed time periods after the detection potential. PAD is commonly done at a gold electrode and since the hydroxylamine oxidation is generally facile at alkaline pH as in the case of carbohydrates [18,19], post-column addition of a strong base (NaOH) was considered. A new quadruple-potential waveform introduced for detection of carbohydrates using PAD was explored for hydroxylamine analysis. The greatest benefit of using the above waveform is the consistent peak area response for hydroxylamine as in the case of carbohydrate applications [20].

To investigate the short-term electrode stability; NH_2OH linear standards were prepared at 2.0, 1.0, 0.5, 0.1 and 0.05 μg/ml concentration ranges per the method. The standards were injected continuously within 1.5 h intervals for about 15 h and peak area responses were recorded. Peak area response of

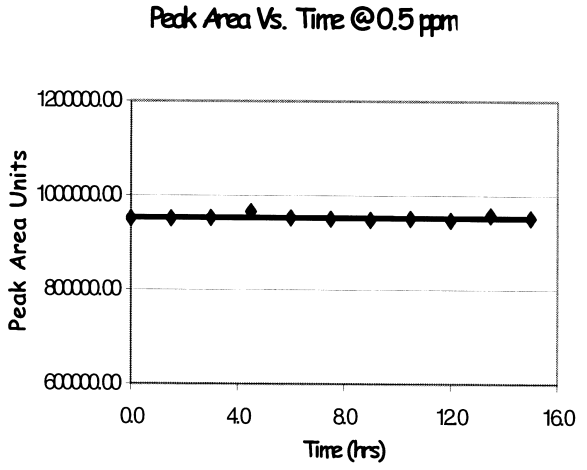

Fig. 1. NH_2OH (0.5 μg/ml) peak area response at Au electrode (15 h) (RSD=0.53%).

hydroxylamine at a gold electrode over a 15 h time period is shown in Fig. 1. The RSDs (n=11) for NH_2OH standards at 0.5 μg/ml (target) level was 0.53% and no apparent decrease in peak area response was noted. The data obtained from hydroxylamine peak area responses at t_0, $t_{7.5\ h}$ and $t_{15\ h}$ for 0.05–2.0 μg/ml concentration ranges are listed in Table 1. For practical purposes, peak area responses at t_0 and $t_{15\ h}$ were compared along with the RSDs of replicate injections. The electrode stability data obtained with the current pulsed amperometric technique were acceptable and very well compared with our previous work with the use of d.c. amperometric detection technique at a Pt electrode as indicated earlier. Hence the quadruple-potential waveform suggested for carbohydrate applications can also be used successfully without any modifications to potential (or time) parameters of the waveform.

3.2. Assessment of the IC method

The objective of this study is to assess the applicability of ion chromatography method for the determination of residual hydroxylamine in waste streams generated from a pharmaceutical reaction process. In order to determine whether a method is suitable for a particular use, the validation data generated using that method must meet pre-determined acceptance criteria. The protocol written for initial assessment of the method (performance characteristics) was based on validation guidelines from our laboratory and was carried out on the IC method. The area responses for the peak of interest were collected and evaluated. Whenever possible, authentic blank samples were used for the validation study. The validation addressed the method linearity, specificity, accuracy/recovery, precision, stability and sensitivity. The experimental design of the validation consisted of a 1-day/one analyst/three replicates per run.

The system suitability (sensitivity) standards consisting of 0.05 μg/ml NH_2OH were injected in triplicate at the beginning and at the end of the run sequence as defined by the method and the protocol. This concentration is equivalent to 0.5 ppm (500 ppb) with respect to sample (final water wash) prepared at 100 mg/ml concentrations specified in the method. The relative standard deviation of peak area responses for six replicate injections was 5.4%, which were well within the previously established system suitability criteria of ≤20%. Stability of the NH_2OH standards was evaluated by monitoring the peak area response throughout the validation experiment. As defined by the draft method and the protocol, NH_2OH bracketing (single point) standard at 0.5 μg/ml concentration was prepared and in-

Table 1
Peak area response at Au electrode (PAD) (~15 h stability study)

NH_2OH conc. (μg/ml)	Peak area (μV s)				RSD (%, n=11)	% Difference: $(t_{15\ h}-t_0)/t_0$
	t_0	$t_{7.5\ h}$	$t_{15\ h}$	Mean (n=11)		
0.0511	86 995.9	75 122.0	76 445.3	80 946.1	5.13	−12.1
0.1022	180 564.5	171 715.8	164 336.4	171 922.3	2.86	−9.0
0.5109	951 367.1	950 871.4	951 870.0	952 981.0	0.53	+0.53
1.0220	1941 468.9	1941 489.5	1941 220.5	1940 803.7	0.20	−0.01
1.9010	3883 294.5	3905 918.3	3867 947.0	3891 256.8	0.30	−0.40

jected throughout the entire run sequence which lasted about 12 h ($n=7$). This concentration is equivalent to 5 ppm (target specification level) with respect to sample preparation described earlier. The RSDs for peak area responses for NH_2OH was 1.1%, which was well within the acceptable target criteria (RSD<10%). There was no significant change in NH_2OH peak area response over the validation run and the solution stability criteria have been met. The results achieved per system suitability criteria are listed in Table 2. To demonstrate the specificity of the method, a mixture of *N*-alkylhydroxylamine analogs at 1.0 μg/ml concentration were prepared in 0.5% ethanol medium which has been used in the process as a clean-up solvent. The resolution between NH_2OH and *N*-methylhydroxylamine is ~2.1. The optimized eluent concentration to achieve the minimal resolution criteria ($R_s>1.5$) per given chromatographic conditions was 11 m*M* H_2SO_4. A representative separation of hydroxylamine analogs in ethanol–water matrix is shown in Fig. 2.

Accuracy and precision of the method were also calculated from the spiked recovery samples prepared at two different NH_2OH concentration levels (0.05 and 0.5 μg/ml). These two levels correspond to limits of quantitation (LOQ) (0.5 ppm) and the target specification level (5 ppm) for NH_2OH with respect to sample concentration (100 mg/ml) as specified in the method. A final water wash sample received from the plant was chosen for this study. Recovery samples were prepared in triplicate at both levels and quantitated against the 0.5 μg/ml NH_2OH bracketing standard. The presence of NH_2OH in the sample matrix has been corrected by quantitating the unspiked samples for NH_2OH levels. The mean recoveries at 0.05 and 0.5 μg/ml NH_2OH levels were 69.4% (100±40) and 93.3% (100±20), respectively. The precisions at 0.05 and 0.5 μg/ml NH_2OH level were 2.8% (RSD<20%) and 1.5% (RSD<10%), respectively. The target criteria have been denoted in the brackets and the precision and accuracy data were well within the target. The recovery data obtained from spiking experiment were listed in Table 3.

Table 2
System suitability

Component	Criteria	NH_2OH Peak area response (μV s)
System suitability ($n=6$)	RSD<20%	81589.5
NH_2OH (0.05 μg/ml)		91014.5
Sensitivity standard		90881.0
		86696.0
		81183.0
		81809.5
		Mean: 85528.9
		SD: 4654.9
		RSD: 5.4%
Stability ($n=7$)	RSD<10%	946638.0
NH_2OH (0.5 μg/ml)		948312.0
Single point standard		958194.0
(~12 h run)		924993.0
		944701.0
		952312.5
		936885.5
		Mean: 944576.6
		SD: 10848.7
		RSD: 1.1%
Resolution	$R_s>1.5$	2.1
Hydroxylamine and *N*-methylhydroxylamine)		

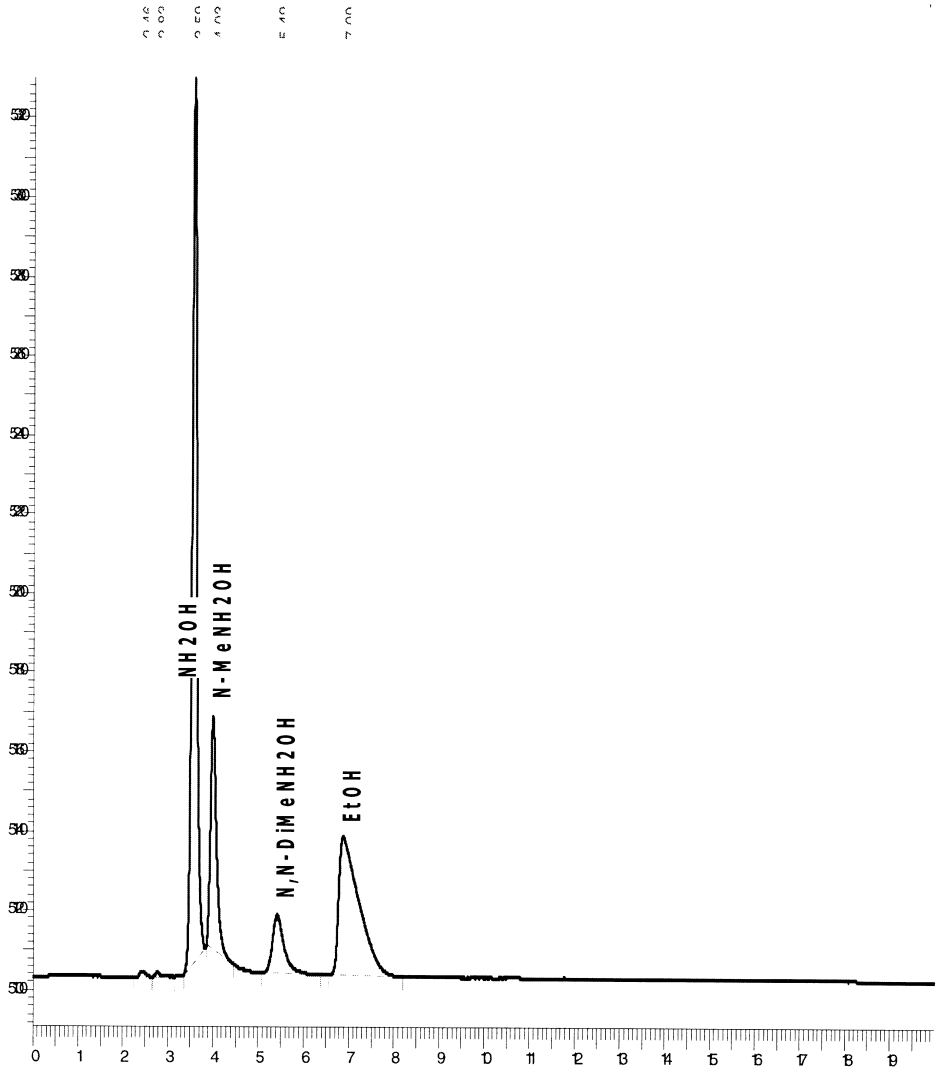

Fig. 2. Chromatogram of hydroxylamine analogs (1 ppm) in EtOH (0.5%) matrix. Time scale in min.

Table 3
Hydroxylamine spiked recovery data (0.05 and 0.5 μg/ml levels)

Component	NH_2OH conc. (μg/ml)		Recovery (%)	Precision: RSD (%)
	Theoretical	Experimental		
Criteria: RSD<20% ±40%	0.055137	0.03728 0.03938 0.03806	69.4	2.8
Criteria: RSD<10% ±20%	0.55137	0.51195 0.52325 0.50848	93.3	1.5

To evaluate the linearity of hydroxylamine responses, six standards of NH_2OH were prepared in Milli-Q water, at concentrations ranging from 0.01 to 2 μg/ml. All linear standards were prepared from 100 μg/ml NH_2OH stock standard solution. To minimize any potential carryover problems and to avoid any detector saturation due to overloading conditions, 2 μg/ml (100 ng on-column) NH_2OH standard was chosen to be the highest linear standard for the given experimental conditions. The

Table 4
Linearity for NH_2OH using STDCRV program

NH_2OH conc. (μg/ml)	Peak area response (μV s)	D-Bias full fit	D-Bias single point at 0.5 μg/ml
0.010034	21761.0	160.4	38.0
0.050170	97225.0	38.3	23.3
0.100339	192170.0	23.4	21.8
0.501695	788594.0	−7.2	0.0
1.003391	1622541.5	−6.0	2.9
2.006782	3542836.0	1.9	12.3
	Corr. Coeff.	0.99878	0.99167
	Slope	1744934	1571900
	Intercept	−23831	0.0
	RMS-X	0.043164	0.111840

STDCRV[1] program was used to assess any significant bias over the linear range as a result of full fit and single fit biases Table 4 illustrates the linearity data. The correlation coefficient of 0.999 was obtained for full fit (multi-point) analysis and 0.992 was obtained for single point fit analysis at the 0.5 μg/ml level. The typical acceptable criteria for correlation coefficient (>0.99) were achieved for all these studies. single point fit analysis at 0.5 μg/ml was performed to see whether 0.5 μg/ml standard can be used to quantitate NH_2OH in the samples without significant biases across the range. This eliminates the need for preparation of multi-point linear standards for typical sample analysis. The %D-Bias for the full fit statistical analysis ranged from 38.3 to 1.9 and single point fit analysis at 0.5 μg/ml ranged from 23.3 to 12.3 for concentration range from 0.05 to 2 μg/ml. The bias over the range 0.05 to 2 μg/ml was <40% for 0.05 ppm and greater levels for single point analysis at 0.5 μg/ml and also for full fit analysis. The protocol criteria for % D-Bias of <40 is acceptable for the lowest range. The higher % D-Bias's for 0.01 μg/ml indicates the method is not linear down to that level. Poor bias at low levels can be due to peak area too close to the intercept. When the intercept is forced through zero, as with the single point fit analysis, the method is accurate down to 0.01 μg/ml. In this case, this is a better representation of the method's accuracy. The method is defined to be linear for concentration ranges that are equivalent to 0.5–20 ppm with respect to sample preparation conditions specified in the method.

The limit of quantitation (LOQ) was defined by the protocol as the lowest concentration that obtained a ±40% D-Bias (acceptable accuracy) with linearity experiments (full fit and single point fit analysis). This criterion was met by the 0.05 μg/ml NH_2OH and the LOQ was best supported by this standard under the given experimental conditions. This is equivalent to 0.5 ppm (500 ppb) with respect to (wrt) sample for practical purposes. The limit of detection (LOD) was defined by the protocol as the concentration of NH_2OH that gives a peak response approximately three times the instrument noise. At the 0.05 μg/ml NH_2OH level the signal-to-noise ratio was approximately 115. Chromatogram for NH_2OH (0.05 μg/ml) sensitivity (LOQ) standard is shown in Fig. 3. Thus an approximate LOD is calculated through interpolation to be 0.0015 μg/ml NH_2OH. This is equivalent to 0.015 ppm (15 ppb) with respect to sample for practical purposes.

3.3. Sample analysis

Sample bottles containing the different washes received from different plants were stored at room condition until analysis. All final wash samples were prepared at 100 mg/ml concentrations in Milli-Q water and quantitated against 0.5 μg/ml NH_2OH

[1]STDCRV is a laboratory-developed personal computer-based statistical software package.

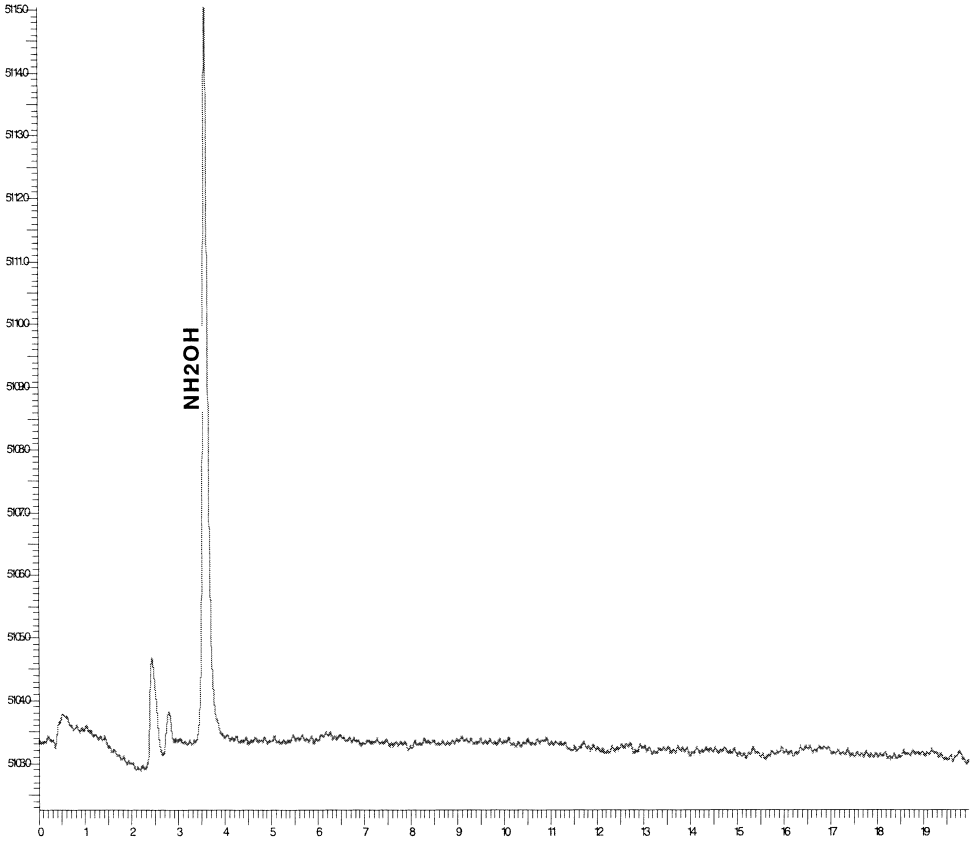

Fig. 3. Chromatogram for 0.05 (μg/ml) NH_2OH sensitivity standard. Time scale in min.

standards injected in a bracketing fashion throughout the injection sequence. All other concentrated samples were diluted appropriately so that the peak area responses for NH_2OH were on scale. In general, no filtrations or pH adjustments were required before prior to sample analysis. Hydroxylamine levels obtained from a typical set of plant samples are listed in Table 5. Each sample was prepared in duplicate and average is reported. A chromatogram for a typical EtOH–water wash sample is shown in Fig. 4.

Table 5
NH_2OH levels obtained from different process streams

Typical plant sample (untreated waste)	NH_2OH (nearest ppm)	NH_2OH (%)
Mother liquor	2033	0.20
Combined filtrate	1118	0.11
First EtOH–water wash	26	0.003
Second water wash	<0.5	–

4. Conclusions

In summary, a direct and rapid ion chromatographic method is described for the determination of trace level hydroxylamine using cation-exchange chromatography with pulsed amperometric detection. The decrease of hydroxylamine peak area response overtime with the application of constant (d.c.) potential at a platinum electrode can be overcome by the quadruple-potential waveform described in this paper. However, post-column addition of a strong base was deemed necessary to facilitate PAD at a gold electrode. The method has been well characterized with respect to linearity, stability, selectivity,

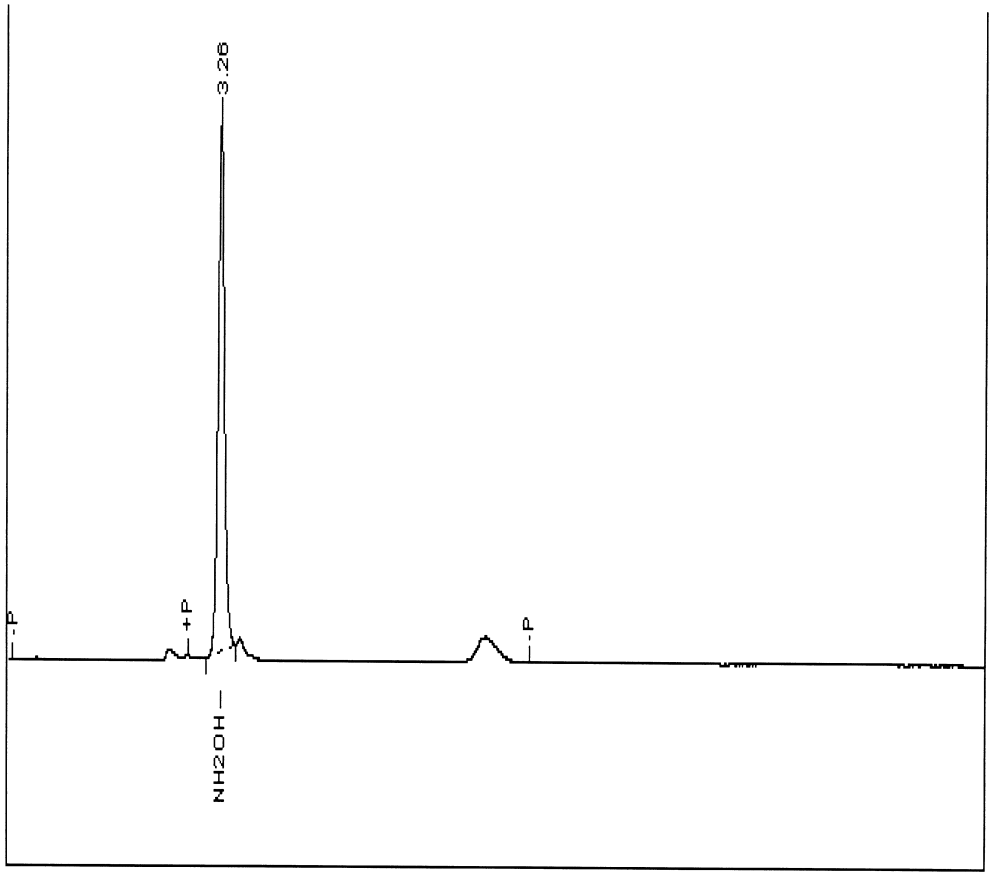

Fig. 4. Chromatograms for a typical EtOH–water wash sample (~15 ppm NH_2OH).

precision and accuracy/recovery. The sensitivity (LOQ~0.5 ppm) attained for the method was well under the required regulatory limit of 5-ppm for hydroxylamine in final waste streams before disposal. It has been successfully applied to waste streams for detection of hydroxylamine generated by a pharmaceutical reaction process.

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