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Determination of trace levels of cyanamide in a novel potassium channel activator bulk drug by pulsed electrochemical detection

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Abstract

Trace amounts of cyanamide may be found in a novel potassium channel activator bulk drug. A chromatographic method is described for detecting trace levels of cyanamide as low as 1 ppm (w/w) using pulsed electrochemical detection at a silver electrode. The bulk drug is dissolved in acetonitrile–water and injected into the IC system. Cyanamide is eluted under isocratic conditions within 10 min.

1. Introduction

Trace amounts of cyanamide may be found in bulk drugs where cyanamide was used in the synthesis of the drug or one of the intermediates. In this report we show a method developed for detecting trace levels of cyanamide in a novel potassium channel activator bulk drug. A sensitive method was needed to determine levels of cyanamide in the bulk drug since cyanamide is toxic and complete removal of it was important from a process development point of view.

Cyanamide detection has been reported using a number of different techniques. A liquid chromatographic method was used to detect calcium cyanamide after pre-column derivatization to dansyl cyanamide [1]. This was used as a stability indicating assay for the analysis of calcium cyanamide in bulk material and dosage form. Cyanamide in plasma was detected selectively and sensitively using another pre-column derivatization technique with 5-(dimethylamino)naphthalene-1-sulfonyl chloride [2]. Chemical assays for cyanamide in biological fluids have been reviewed [3]. Further studies include: the spectrophotometric determination of cyanamide with pentacyanoferrates [4], a gas chromatographic method to detect cyanamide in blood plasma after extraction and derivatization with heptafluorobutyric anhydride (HFBA) [5], a reaction rate method for determining trace concentrations of cyanamide [6], use of an ion selective electrode for cyanamide [7], an infrared method to determine trace quantities of cyanamide in guanidine sulfate [8], indirect argentometry to detect cyanamide in solutions [9], analysis of cyanamide derivatives by the separation of mixtures on ion-exchange resins [10], and use of *p*-phenylenediamine hydrochloride for the spectrophotometric determination of cyanamide [11]. Many of these methods are tedious since they involve derivatization of some sort.

In this paper, a direct chromatographic method using pulsed electrochemical detection

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(PED), on a polymeric AS-10 anion-exchange column, is described for sensitive determination of cyanamide in a bulk drug substance.

The pulsed electrochemical detector is equipped with a pair of silver working electrodes. Silver enables selective detection of the cyanide moiety in the cyanamide molecule with extremely high sensitivity at a very low oxidation potential (+0.08 V) according to the reaction:

$$Ag^0 + (H_2NCN)^- \rightleftharpoons Ag^+(CNNH_2)^-$$

This chromatographic method with PED can monitor cyanamide down to ppm (w/w) levels in a bulk drug substance as shown in this study.

2. Experimental

2.1. Instrumentation

A Dionex gradient pump module (GPM) and eluent degas module (EDM-II) and Dionex pulsed electrochemical detector (PED-1) equipped with silver working electrodes were controlled through a Dionex interface (Dionex, Sunnyvale, CA, USA) by a Compaq Deskpro 386/25L personal computer running the AI-450 software version 3.30 (Dionex). The injector used was Hitachi autosampler Model 655A-40 (Hitachi Instruments, Danbury, CT, USA).

2.2. Reagents

Sodium hydroxide 50% (w/w) was purchased from J.T. Baker (Phillipsburg, NJ, USA). 200 m*M* Sodium Hydroxide was prepared from this solution. HPLC grade acetonitrile (MeCN) was obtained from Baxter Scientific (McGaw Park, IL, USA). Deionized water was further purified using a Millipore Milli-Q system (Millipore, Bedford, MA, USA). All mobile phases were filtered before use with 0.45-μm Nylon-66 filters (Schleicher & Schuell, Keene, NH, USA) and a solvent filtration kit (Schleicher & Schuell). Hydrochloric acid (concentrated) was purchased from J.T. Baker from which a 1-*M* solution was prepared for column clean-up. Cyanamide 50% (w/w) was obtained from Aldrich (Milwaukee, WI, USA) and was used for standard preparations.

2.3. Chromatographic system

An IonPac AS-10 anion chromatographic column with an IonPac AG-10 anion guard column (Dionex) was used. Mobile phase [MeCN–50 m*M* NaOH (1:99)] was prepared by transferring 500 ml of 200 m*M* sodium hydroxide and 20 ml acetonitrile into a 2-l volumetric flask and diluting to volume with water. The flow-rate was 1 ml/min. The column temperature was ambient. Under these conditions cyanamide elutes in under 10 min.

2.4. Detector conditions

Detection was performed with a Dionex pulsed electrochemical detector using silver electrodes. Range on the detector was 300 nC. Data sampling was done only from 0.1 to 0.78 s (one pulsing cycle is 0.88 s). After that, a positive pulse at 0.1 V and a negative sweep at −0.1 V cleaned the electrode surface.

Data collection was performed on a chart recorder (Kipp & Zonen, Delft, Netherlands) or an IBM-PS/2 computer with an advanced computer interface (Dionex) and AI-450 software (Dionex). The A1-450 software also controlled the Dionex instrument and the gradient program. Data was simultaneously collected on a Micro VAX 3400 microcomputer running VG Multichrom software version 1.8 through a VG chromserver (VG Instruments, Danvers, MA, USA).

2.5. Preparation of standards and samples

Sample solvent was prepared by separately measuring equal amounts of MeCN and water, combining them in a suitable container and mixing well. Stock solution 1 (1000 μg/ml cyanamide) was made by accurately weighing 100 mg 50% cyanamide into a 50-ml volumetric flask containing 25 ml of the sample solvent. It was diluted to volume with the sample solvent and

mixed well. From this stock solution working standard solution 1 (100 ng/ml cyanamide), working standard solution 2 (50 ng/ml cyanamide) and working standard solution 3 (15 ng/ml cyanamide) were made by appropriate dilutions with the sample solvent.

Working sample solution (5 mg/ml of the bulk drug) was prepared by transferring 50.0 mg of the bulk drug sample into a 10-ml volumetric flask. A volume of 5.0 ml of MeCN was transferred to the flask and after sample dissolution diluted to volume with water.

3. Results and discussion

Fig. 1 shows the chromatogram of the working standard solution 3 containing cyanamide at 15 ng/ml. The retention time of cyanamide is 6.99 min and the k' was calculated to be 1.33. The pulse feature of PED provides baseline stability due to the clean electrode surface resulting in better reproducibility compared to continuous DC amperometry. Fig. 2 shows the chromatogram of a blank injection (sample solvent). A very small peak is seen in the blank at the retention time of cyanamide which calculates to be less than 1 ppm (the detection limit). Fig. 3 shows the chromatogram of the bulk drug substance stored at 30°C and 75% relative humidity. Fig. 4 shows the chromatogram of a sample after the synthetic process had been optimized. The process has been optimized so that the levels of cyanamide in typical batches of the potassium channel activator are less than the detection limit (1 ppm w/w) as shown in Fig. 4.

One of the main advantages of this technique is that no sample clean up is necessary due to the selectivity. In this instance, bulk drug substance is dissolved in acetonitrile–water (50:50) and directly injected into the ion chromatography (IC) system.

The linear regression data for cyanamide is shown in Table 1. Linearity standard solutions were injected over the range of 15 to 150 ng/ml. Peak areas were used for regression analysis. Within this range, cyanamide had a linear response, with a correlation coefficient of 0.9982. A three-point calibration is used for the quantitation of cyanamide. The three standards used here cover the range of 15–100 ng/ml (3–20 ppm).

Accuracy for cyanamide was determined by spiking a batch of the bulk drug substance at various levels with cyanamide and calculating the recovery (Table 2). Spiked sample solutions were prepared by adding 0–25 ppm cyanamide to the bulk drug substance solution. The re-

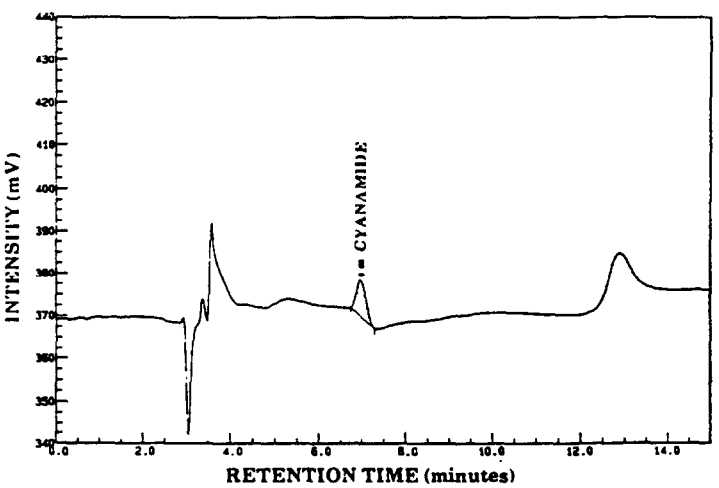

Fig. 1. Chromatogram of the working standard solution 3 showing cyanamide at 15 ng/ml (3 ppm for the drug concentration at 5 mg/ml).

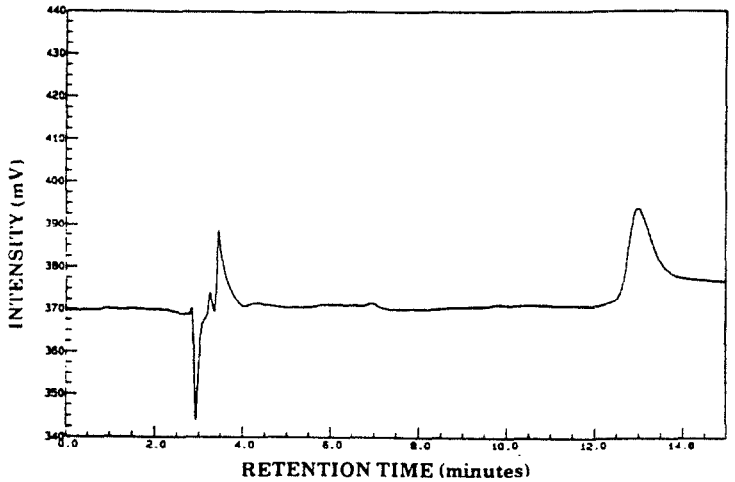

Fig. 2. Blank injection of the sample solvent, acetonitrile–water (50:50).

covery of cyanamide was determined by

$$\% \text{ Recovery} = \frac{\text{ppm found}}{\text{ppm spiked}} \cdot 100$$

The recovery of cyanamide was satisfactory. The recovery values for cyanamide spikes ranging from 3 to 25 ppm were 89 to 106%.

The precision of the system for cyanamide was determined by injecting the bulk drug substance at 5 mg/ml several times. An investigational batch containing 5 ppm (w/w) of cyanamide was used for this study. Peak areas of multiple injections of this same solution were used to calculate the precision of the system. The standard deviation was 0.25 for a mean value of 5.4 ppm and the coefficient of variation was 4.6% as shown in Table 3.

The intra-day reproducibility of the method

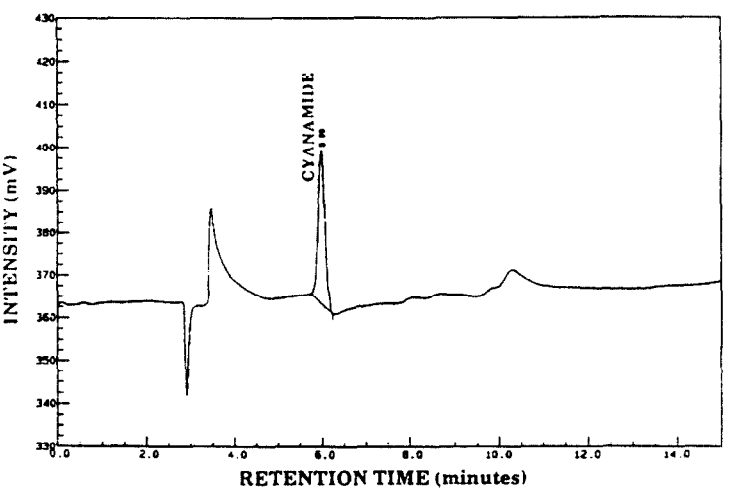

Fig. 3. Chromatogram of a batch of a highly stressed stability sample of the bulk drug substance.

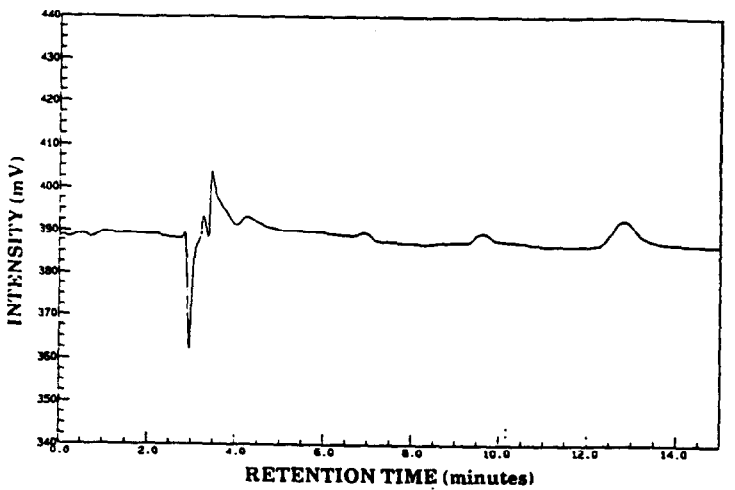

Fig. 4. A typical batch of the drug substance at the working concentration, 5 mg/ml.

Table 1
Linear regression for cyanamide

Concentration (ng/ml)	Area 1	Area 2	Mean peak area
14.99	147 460	140 702	144 081
24.99	218 601	204 770	211 686
49.85	381 401	363 189	372 295
74.78	521 410	512 321	516 866
99.70	648 182	657 579	652 881
124.6	766 612	788 738	777 676
149.6	879 904	909 206	894 555

$y = 5594x + 79\,670$; $r = 0.9982$.

Table 2
Recovery of cyanamide from the bulk drug substance

Added (ppm)	Found (ppm)	(Recovery (%)
0	<DL ($n = 4$)	–
2.99	2.76	92
4.99	5.06	101
9.98	10.62	106
14.96	15.51	104
19.95	18.82	94
24.94	22.26	89

for cyanamide was determined by weighing the bulk drug substance eight different times and analyzing the results using this method. The relative standard deviation was 6.4% for a mean value of 5 ppm and the standard deviation was 0.34. These results are shown in Table 4.

The inter-day reproducibility of the assay was demonstrated for three batches assayed on two days. Samples analyzed on day 1 gave compar-

Table 3
Precision of the system for cyanamide

Injection	Peak area	Cyanamide (ppm)
1	211 806	5.25
2	203 849	5.02
3	206 431	5.10
4	218 506	5.44
5	213 626	5.30
6	215 071	5.35
7	229 927	5.77
8	212 730	5.28
9	224 656	5.62
10	226 801	5.68
Mean	216 340	5.38
S.D.	8153	0.25
R.S.D. (%)	3.8%	4.6%

Table 4
Reproducibility of the method for cyanamide

Sample mass (mg)	Cyanamide found (ppm)		
	Injection 1	Injection 2	Mean
49.8	6.00	5.47	5.85
50.0	5.52	5.72	5.62
50.1	4.80	5.49	5.15
49.7	5.21	4.95	5.08
49.9	4.99	4.94	4.97
50.0	4.46	5.47	4.97
50.2	5.41	4.76	5.09
49.9	6.11	5.07	5.59
Mean			5.29
S.D.			0.34
R.S.D. (%)			6.4

able results on day 2, three weeks later. The results are shown in Table 5.

The stability of the spiked bulk drug substance working sample solutions (apparent pH *ca.* 5.4) also was investigated over a period of 53 h (*ca.* 2 days). No significant increase in cyanamide content was found in the sample solvent as shown in Table 6.

4. Estimation of detection limit (DL) and minimum quantifiable limit (MQL)

The detection limit (DL) and the minimum quantifiable limit (MQL) for cyanamide were estimated as a function of the standard deviation of the baseline noise and the slope of the linear regression line. The baseline noise was estimated from the standard deviation of the peak area of ten replicate injections of a batch containing *ca.* 5 ppm cyanamide (precision of the system study).

Table 5
Day-to-day reproducibility of the method

Batch number	Cyanamide (ppm)	
	Day 1	Day 2
3 138 216 823	11	11
3 138 216 727	5	4
3 138 216 427	11	11

Table 6
Stability of the bulk drug substance in the sample solvent

Period (h)	Cyanamide found (ppm)
4	11.50
7	12.30
26	13.43
29	12.54
53	13.31
	Mean = 12.62
	S.D. = 0.79
	R.S.D. = 6.26%

The DL and MQL values for cyanamide were estimated as follows:

$$\text{DL (ppm)} = \frac{3 \cdot \text{S.D.} \cdot 10^6}{S \cdot C_w}$$

$$\text{MQL (ppm)} = \frac{10 \cdot \text{S.D.} \cdot 10^6}{S \cdot C_w}$$

where: S.D. is the standard deviation of the baseline noise, estimated from the replicate injection reproducibility (area counts); 10^6 is a factor to convert to ppm; S is the sensitivity, *i.e.*, slope of the response *versus* concentration, determined from standard linearity evaluation (area counts per ng/ml); C_w is the working concentration ($5 \cdot 10^6$ ng/ml).

It has been observed that a DL of 1 ppm and a MQL of 3 ppm are reliably attainable for cyanamide, showing reasonable agreement with statistical calculations. Therefore, these values for DL and MQL are used for reporting purposes.

Fig. 5 is a chromatogram of the drug substance spiked with cyanamide at the DL (1 ppm). Fig. 6 is a chromatogram of the drug substance spiked at the MQL (3 ppm).

5. Conclusions

A highly selective ion chromatographic meth-

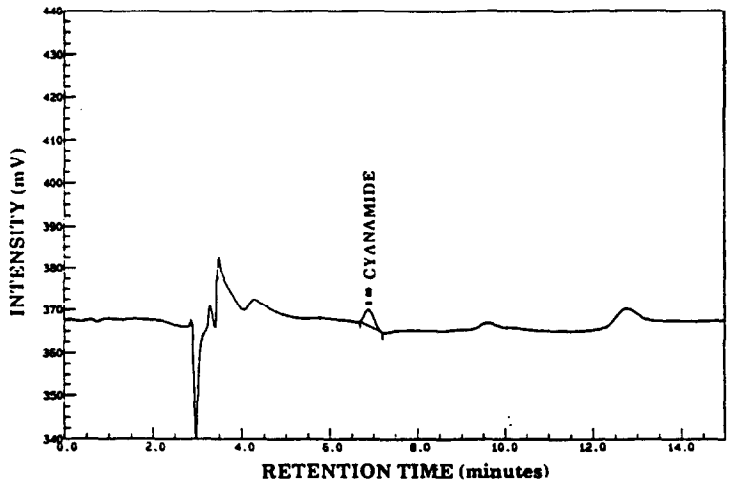

Fig. 5. Detection limit of cyanamide at 5 ng/ml (1 ppm) in the presence of the bulk drug substance at 5 mg/ml.

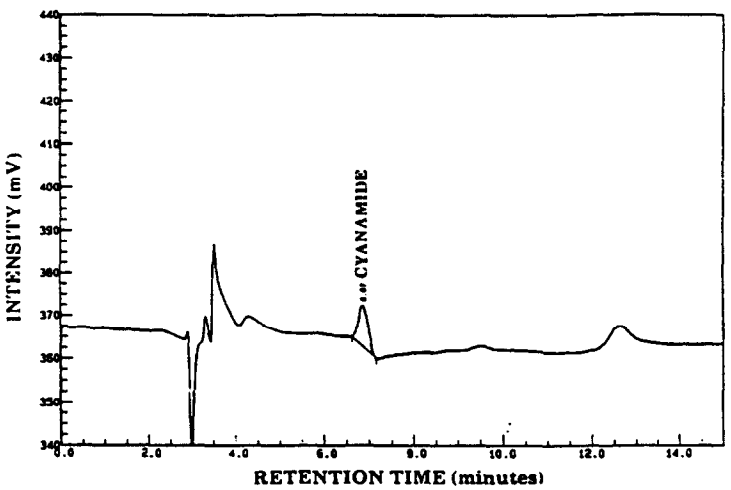

Fig. 6. Minimum quantifiable limit of cyanamide at 15 ng/ml (3 ppm) in the presence of the bulk drug substance at 5 mg/ml.

od with pulsed electrochemical detection (PED) was developed for the sensitive detection of ultratrace levels of cyanamide (1 ppm, w/w) in a novel potassium channel activator bulk drug substance.

Acknowledgement

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