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Short communication

Analytical method for the quantitative determination of cyanuric acid as the degradation product of sodium dichloroisocyanurate in urine by liquid chromatography mass spectrometry

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Abstract

A simple and selective analytical method for the quantitative determination of cyanuric acid, the degradation product of sodium dichloroisocyanurate (NaDCC), in human urine is reported herein. The sample preparation involved the use of diatomaceous earth extraction columns. Quantification was achieved by liquid chromatography mass spectrometry using negative ion electrospray with a cyano (CN) column. Between day relative standard deviation less than 10% ($n = 6$) was obtained at the 5 mg L^{-1} level. The assay was linear over the investigated range 0–20 mg L^{-1} and the limit of detection (LOD) was confirmed to be 0.1 mg L^{-1}. The method was applied to monitoring levels of cyanuric acid in healthcare workers using disinfectants products containing NaDCC.

Keywords: Cyanuric acid; Liquid chromatography; Mass spectrometry; Biological monitoring; Urine

1. Introduction

Sodium dichloroisocyanurate (NaDCC) is a chlorinated derivative of cyanuric acid. NaDCC is widely used as an ingredient in formulations for scouring powders, household bleaches, dishwasher compounds, general sanitisers, and for swimming pool disinfection [1]. In particular, NaDCC as a disinfectant has advantages when dealing with body fluid spills which can present a serious potential hazard to both patients and the staff who are responsible for cleaning and disinfecting the area. Traditionally, such spills have been treated with disinfectants such as hypochlorite solutions. However, hypochlorite is inactivated by organic material [2] and is therefore ineffective as a disinfectant for spills of body fluids. In contrast, NaDCC has been found to be more efficient in disinfecting body fluids than hypochlorite and has proven effective against bacteria, fungi and viruses [3]. The relatively recent introduction of NaDCC into commercial disinfectants has meant that the opportunity to gather real occupational exposure data has so far not been presented.

When dissolved in water NaDCC rapidly hydrolyses to form cyanuric acid and releases free available chlorine in the form of hypochlorous acid. The hydrolysis is an equilibrium reaction, and at low (mg L^{-1}) concentrations it is dissociated to a large extent. Thus, cyanuric acid is the primary substance of toxicological and environmental interest when monitoring chlorinated cyanurates in humans. Health effects associated with exposure to NaDCC could include the combined toxicological effects of chlorine and cyanuric acid. The toxicity of NaDCC and cyanuric acid have been extensively studied and summarised [4]. Although cyanuric acid was found to be no more than slightly toxic, guidelines have been set for tolerable daily intake (TDI) for NaDCC from treated drinking water at 0–2 mg kg^{-1} of body weight per day [5].

Several methods to determine cyanuric acid have been recently reviewed [6]. The majority of the methods utilise reversed phase HPLC, silica columns and phosphate buffers with improved variations using these methods reported recently [6–9]. Although the HPLC methods are simple and rapid, they use spectrophotometric detectors operating at 200 nm where other interferences can absorb, especially when dealing with biological matrices. Alternatively, additional selectivity can be gained from using mass spectrometry (MS). Cyanuric acid has

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been analysed in urine by MS using a solid probe [10], gas chromatography MS [11] and recently by stable association complex electrospray mass spectrometry [12]. The objective of this study was to develop a selective, sensitive and straightforward method for the trace determination of cyanuric acid in urine and to apply the method to healthcare workers, to establish the potential risk of exposure through inhalation, ingestion or dermal absorption during normal use of NaDCC solutions.

2. Experimental

2.1. Chemicals, standard and sample preparation

Cyanuric acid (purity > 98.0%), maleic acid, methanol, ammonium formate and methyl-*tert*-butyl ether (MTBE) (analytical reagent grade) were all purchased from Fisher Scientific (Loughborough, UK).

A stock standard solution of cyanuric acid (1 mg mL^{-1}) was prepared in methanol by sonication at 35 °C for 25 min. Working standard solutions from the stock solution, containing 100 and 10 mg L^{-1} of cyanuric acid, were prepared in methanol by serial dilution for use as spiking solutions. The spiked blank urine (2 mL) used for the calibration curve was prepared at concentrations of 0, 0.5, 1.0, 2.5, 5, 10 and 20 mg L^{-1}. For estimation of recovery/quality control (QC), blank urine (2 mL) was spiked at the 5 mg L^{-1} of cyanuric acid. An unspiked sample of the same urine was kept as blank and swimming pool water (2 mL) from outdoor pools in the UK that use cyanuric acid was analysed for comparison.

2.2. High performace liquid chromatography

The HPLC system comprised of an Agilent Technologies (Palo Alto, CA, USA) 1100 binary series LC pump, equipped with a vacuum degasser, and an autosampler. For LC–MS a guard column Genesis C18 2 cm by 4 mm I.D. (4 μm) was used (Jones Chromatography, Hengoed, UK) and the analytical column was a CN (Zorbax®) 250 mm by 4.6 mm I.D. and 7 μm particle size (Agilent Technologies, Palo Alto, CA, USA). The mobile phase was MeOH:aqueous 1 mM ammonium formate (89:11, v/v) pH 7.4. An isocratic separation was used with the column at a flow rate of 0.3 mL min^{-1}. Sample volume of 1 μL was used for all LC–MS experiments.

2.3. Mass spectrometry

The HPLC system was directly connected to an Agilent Technologies LC/MSD XCT ion trap mass spectrometer, equipped with an orthogonal electrospray ion source. Cyanuric acid was detected in negative electrospray ionisation mode using the following parameters: nebulizer pressure 103.4 kPa, drying gas flow rate 6 L min^{-1}, dry gas temperature 350 °C, trap drive 32.2 V, capillary exit −100.6 V, skimmer −40.0 V, scan range 50–1500 amu, dwell time 100 ms and an ICC target of 35000 counts. Cyanuric acid (MW 129) was determined by monitoring $[M-H]^-$ ions at *m/z* 128.

2.4. Extraction procedure and clean-up

Aliquots of urine and swimming pool water (2 mL), spiked blank urine for the calibration curve, quality control (QC) and volunteer urine samples, were transferred into polypropylene tubes (12 mm × 75 mm). To all the samples (blank, spiked, volunteer and water) 250 μL of 20 mg mL^{-1} maleic acid solution was added. The sample preparation involved the use of unbuffered diatomaceous earth extraction columns (ChemElut® 1003, Varian, Poole, UK). All of the sample contained in the polypropylene tubes was applied to the cartridge and after urine percolation (3.5 min) the analyte was eluted with MTBE (6 mL) and after an interval of 3.5 min a further aliquot MTBE (6 mL) was applied. After 15 min of the second addition of MTBE the pooled eluent was evaporated to dryness at ambient temperature under a gentle stream of nitrogen and reconstituted in 100 μL of MeOH for LC–MS analysis. Methanol was used to reconstitute the extracts because it showed a better peak shape and an enhanced separation of cyanuric acid from an interfering matrix peak compared to reconstitution in mobile phase.

2.5. Stability of cyanuric acid in urine

The stability of cyanuric acid in urine was determined by analysing spiked urine at 10 mg L^{-1} in plastic tubes. The tubes were kept at ambient temperature (shielded from light), cool conditions (<4 °C) or at temperatures of below −10 °C. The urine extracts were analysed at day 0, 1, 2, 3, 4, 10, 16 and 28.

3. Results and discussion

3.1. Sample preparation

Initially a liquid:liquid extraction, previously reported by Magnuson et al. [12] was evaluated. Briefly, 1 mL of urine, pH adjusted with sulphuric acid to <6 was shaken/extracted with 3 mL MTBE. The extract was evaporated and reconstituted in methanol. However, the addition of sulphuric acid required for charge neutralisation of cyanuric acid gave a low ES–MS response compared to a spiked water extract at the same concentration (10 mg L^{-1}). Other acids investigated were formic and acetic which also resulted in a similar low response. When liquid-liquid extractions were carried out with no acid, higher responses were obtained but repeatability for a set of spiked solutions of water was poor. Subsequently other organic acids such as malonic, maleic and barbuturic were investigated. Of these maleic acid (pK_a 1.92) was able to adjust 1 mL of urine to a pH <6 and allowed high recovery of cyanuric acid from spiked water and urine extracts. The recovery of cyanuric acid was found to be related to the concentration of maleic acid up to 3 mg mL^{-1}. To improve the detection limit, 2 mL of urine was adjusted with maleic acid and extracted with 6 mL of MTBE but the efficiency was only ∼50% and of a similar scale when 10 mL of MTBE was used.

The use of solid phase extraction (SPE) was investigated and initially focussed on carbon columns but they did not retain cyanuric acid consistently when applied in urine

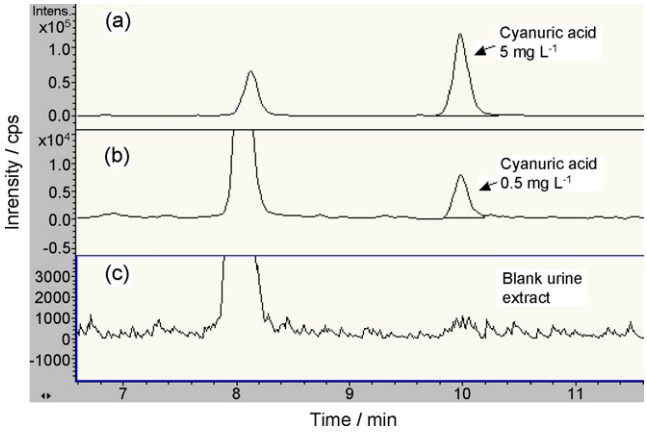

Fig. 1. Extracted ion chromatograms (a) urine extract spiked at 5 mg L^{-1}; (b) urine extract at the lowest calibrated level of 0.5 mg L^{-1} and (c) blank urine extract. Retention time of cyanuric acid is 10.0 min.

matrix and therefore was not pursued further. In contrast ChemElut® cartridges (diatomaceous earth), which allow a modified liquid–liquid extraction to take place in a SPE cartridge format, were employed successfully in recovering nearly all of the cyanuric acid with maleic acid and using 12 mL of MTBE as an elution solvent.

3.2. Selectivity and method performance

The chromatographic conditions were optimised to give adequate separation of cyanuric acid from an interfering peak, which remained after clean-up of the urine extract. The interfering peak was resolved when using a cyano phase column of 250 mm length by 4.6 mm id. The cyano column also allowed a lower limit of detection when compared with C8 and C18 columns of equal dimensions because of a narrower peak width. A low flow rate was used for the column I.D. in this study to allow compatibility with the ES source, but because of the high organic content of the mobile phase peak widths were comparable to reverse phase strategies. A further reduction in retention time could be achieved, not reported in this work, by scaling down the column length and particle size whilst using the same stationary phase. Fig. 1 shows the extracted ion chromatograms for the acquisition method of a spiked extract, lowest calibrated level and blank urine showing clear separation from the matrix interfering peak.

Table 1 gives a summary of regression parameters for calibration curves, which were linear over the investigated range 0–20 mg L^{-1}. Results for blank urine extracts spiked at 5 mg L^{-1} are shown in Table 2. Overall, the inter- and intra-assay RSDs are low demonstrating good precision, repeatability and reproducibility of the method. In addition, the mean spiked recovery values for inter- and intra-assay are close to the spiked level of 5 mg L^{-1}.

Table 1
Summary of linear regression parameters for calibration curve and limits of detection for determination of cyanuric acid in urine

Assay's parameters	Values
Dynamic range (mg L^{-1})	0–20
Correlation coefficient (r)	0.9993
Determinations (n)	6
Slope ± S.E. (mg L^{-1})	11.61 ± 0.1023
Intercept ± S.E.	1.83 ± 0.0040
LOD (mg L^{-1})	0.1

The LOD, defined as the amount injected which gave a signal equivalent to three times the baseline noise, was determined experimentally at 0.1 mg L^{-1}. This is comparable to the method of Allen et al. [10], the only previously reported method for analysing urine samples from exposed persons by HPLC, when taking into account equivalent concentration factors. However, the current method allows a four-fold increase in concentration (2 mL to 100 μL) because matrix factors are not a limitation and this results in an overall five-fold improvement in LOD. In addition, the method is much more straightforward using only a single extraction procedure compared to the double extraction.

3.3. Sample stability

The stability study looked at spiked urine samples ($n = 3$) and a spiked aqueous blank over a period of 28 days at ambient ~22 °C (in darkness), <4 °C (refrigerated) and at ~−20 °C (frozen). At room temperature the cyanuric acid spiked sample was stable (within the accepted analytical variation of 9.5%) until day 3; by day 10 it had degraded to about 50% of the original spiked value. The refrigerated sample was still within the accepted analytical variation by day 16 although showing a degrading trend. The stability of cyanuric acid in urine under frozen conditions was not affected for 28 days. A contribution from the other matrix components of the urine is likely in the degradation of cyanuric acid.

3.4. Application

The method was applied to the analysis of urine samples collected from healthcare workers using NaDCC based disin-

Table 2
Within batch- and between batch-assay relative standard deviations and system reproducibility

	Cyanuric acid		
	Within batch repeatability ($n = 10$)	Between batch reproducibility ($n = 6$)	Overall system reproducibility ($n = 60$)
x (mg L^{-1})	4.8	5.3	5.3
SD	0.5	0.3	0.5
RSD (%)	10.5	6.0	9.5

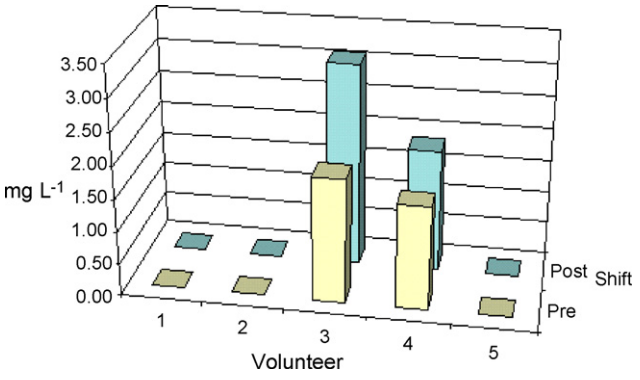

Fig. 2. Exposure data for post- and pre-shift collection of urine for five healthcare workers using NaDCC disinfectant solutions at 1000 mg L^{-1}.

fectant products. Samples were taken pre- and post-shift where workers were using disinfectant solutions of a concentration of approximately 1000 mg L^{-1}. Of the workers, only one person prepared the solutions (by adding powder to a bucket of water) whilst all healthcare workers participated in using the solution to disinfect the hospital beds and surrounding areas. To provide a comparison, samples collected from volunteers not known to be exposed to NaDCC, cyanuric acid or swimming pool water were also analysed. Fig. 2 shows that generally healthcare workers are only exposed to a small amount of cyanuric acid when applying the NaDCC disinfection product. Of the volunteers 1 and 5 prepared the solutions and the results show that participation in the preparation of the solution does not necessarily lead to exposure. On the other hand, volunteers 3 and 4 were observed to undertake cleaning tasks above their heads, which could result in possible ingestion of the solution or run-off down the arms. The same volunteers also showed small levels in their urine prior to the disinfection routine, which might be due to previous exposure to cyanuric acid as this was a routine that they performed every day. Overall the results show minimal exposure to healthcare workers using 1000 mg L^{-1} NaDCC solutions. In comparison a previous study showed cyanuric acid exposure levels up to 8.8 mg L^{-1} from swimmers in swimming pool water for 2 h [10]. The samples from volunteers not associated with any exposure did not show any levels of cyanuric acid. Three samples of swimming pool water contained 16, 9 and 34 mg L^{-1} of cyanuric acid, respectively (sample diluted to bring them into the calibration range) and the results show levels that are similar to those reported by other authors analysing cyanuric acid in swimming pool water [6,7].

4. Conclusion

The results of the experiments show that the LC–MS method is capable of providing a rapid, selective and reproducible determination of cyanuric acid in urine and water. The assay is sensitive enough to detect low concentrations needed when considering monitoring people who are at risk of occupational exposure. In addition, the assay can be used to determine cyanuric acid in swimming pool water and in urine obtained from swimmers.

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