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Cyanide detection in gastric juice with corrin-based chemosensors

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This technical note describes the detection of cyanide in artificial gastric juice with corrin-based chemosensors. The application of this method in authentic human samples is demonstrated in a proof-of-principle forensic investigation.

1. Introduction

Cyanide (CN^-) is highly toxic to humans and most forms of life because of its strong binding to biologically active transition metal ions.[1,2] In particular, it effectively blocks the vacant coordination sites of ferri-(Fe^{III}) containing heme complexes and thereby inactivates cellular respiration.[3–5] Cyanide intoxications may occur from intentional and accidental cyanide uptake and are most frequently encountered in industrialized countries after smoke inhalations from fires.[6] Indeed, more than 80% of fire victims die from smoke gas intoxications and not from burns.[7] One of the most toxic combustion products of nitrogen containing organic products is hydrogen cyanide (HCN; pK_a = 9.04 (ref. 8)),[9] the conjugated acid of cyanide. Testing for cyanide intoxication is therefore required in clinical diagnostics and forensic investigations.[1,2,10,11] For this purpose, different types of body fluids such as blood,[12–17] saliva[18,19] and gastric acid[20,21] are usually analysed. Most methods require sample pre-treatment, sophisticated technical equipment (*e.g.* GC-MS,[22] ESI-MS-MS[23]) as well as specialised users and hence, the analysis of cyanide is not yet routine in hospital and forensic settings.[2] Recent developments indicate that low-cost and straightforward test-kits with colorimetric and fluorometric indicators may overcome these limitations.[12,17,24–29] In this context, vitamin B_{12} based chemosensors are currently representing one of the most promising systems for detecting cyanide in biological samples in terms of handling, selectivity, sensitivity and response time.[4,26,28–34] Based on earlier pioneering investigations of our group,[34–37] we describe herein the detection of cyanide in artificial gastric juice with corrin-based chemosensors and solid phase extraction (SPE).[24,38] The applicability of the method is demonstrated in a proof-of-concept forensic investigation.

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2. Materials and methods

2.1 Materials and general information

Chromabond C18ec polypropylene columns (100 mg), Chromafix C18ec columns (s) (270 mg), shorty (5 mg) and Chromabond adapter PP were obtained from Macherey-Nagel AG, Switzerland. The columns were pre-conditioned prior use with MeOH (1 mL) and water (5 mL).

UV-vis spectra were measured at $T = 21 \pm 1$ °C with a Cary 50 spectrometer (Varian, Switzerland) using quartz cells with a path length of 1 cm.

2.2 Preparation of solutions

The desired pH value of the stock solution of the buffer Ches (0.25 mol L^{-1}; pH 9.6) was adjusted by the addition of 2 mol L^{-1} NaOH and 1 mol L^{-1} HCl solutions. The pH values were measured with a Metrohm 827 pH lab (Metrohm, Switzerland).

The cyanide stock solution (10^{-3} mol L^{-1}) was freshly prepared every day.

Artificial gastric juice (AGJ) was obtained by dissolving pepsin (32 mg, 89 μmol), NaCl (20 mg, 0.34 mmol) and HCl (32%, 0.07 mL) in 10 mL water.[39]

2.3 Preparation of the chemosensor aquacyanocobyrinic acid heptamethyl ester (ACCbs)[40]

The corrin-based chemosensor aquacyanocobyrinic acid heptmethyl ester (ACCbs) was synthesized as described elsewhere.[36,40]

2.4 Artificial gastric juice cyanide detection

Artificial gastric juice (AGJ; 500 μL) was spiked with cyanide ($[CN^-]$ = 0–160 μmol L^{-1}). Fifteen minutes later, the gastric juice sample was diluted to 972 μL with Ches buffer (0.25 mol L^{-1}; pH 9.6; 392–472 μL, depending on the volume of CN^- added) and ACCbs (28 μL; 1.5 mmol L^{-1}; 42 nmol) was added.

Cyanide was either detected directly in solution (A) or after solid-phase extraction (B).

A: The solution was transferred to a quartz cuvette and analyzed with UV-vis spectroscopy.

B: After one minute, the sample was passed through a preconditioned C18ec column and subsequently washed with water (3 mL) to remove adhering gastric juice from the C18ec material. The chemosensor was subsequently eluted from the C18ec column with MeOH (0.4 mL). The eluate volume was adjusted to 0.5 mL with MeOH, transferred to a quartz cuvette and analysed with UV-vis. The cyanide content was determined with the help of a calibration curve.

2.5 Calibration curves

Homogenous conditions. UV-vis spectra of AGJ samples spiked with different amounts of cyanide ($[CN^-] = 0$–160 μmol L^{-1}) were analysed. The calibration curve was obtained by plotting the absorptions at 583 nm against CN^- concentrations. Each experiment was repeated three times to assign mean values and standard deviations. A linear equation ($y = a + bx$) was obtained by fitting the linear range with OriginPro2015.

Solid-phase extraction. UV-vis spectra of AGJ samples spiked with different amounts of cyanide ($[CN^-] = 0$–160 μmol L^{-1}) were analysed after eluting the reagent from the C18ec cartridge with MeOH ($V = 0.4$ mL). The eluate volume was adjusted to 0.5 mL with MeOH and the UV-vis spectra were recorded. The calibration curve was obtained by plotting the absorptions at 583 nm against CN^- concentrations. Each experiment was performed three times in order to determine the mean value and standard deviations. A linear equation ($y = a + bx$) was obtained by fitting the linear range with OriginPro2015.

2.6 Artificial gastric juice cyanide detection at different incubation times

The influence of incubation time (defined as time between spiking and analysis) was determined for AGJ samples stored at room temperature ($T = 23 \pm 1$ °C). Samples spiked with cyanide ($V_{sample} = 0.5$ mL, $[CN^-]_{AGJ} = 40$ μmol L^{-1}) were analysed after different time intervals (t = 20–1470 min) and analysed as described under Section 2.4. Each experiment was repeated three times to assign mean values and standard deviations. In the same way, experiments were performed for AGJ samples ($V_{sample} = 0.5$ mL, $[CN^-]_{AGJ} = 40$ μmol L^{-1}) that were stored for different time intervals (t = 1–55 d) at −21 °C.

2.7 Gastric juice cyanide detection in authentic human samples

Cyanide was monitored in diluted human gastric juice samples (HGJ) with (A) and without (B) solid-phase extraction using corrin-based chemosensors. In addition, the samples were analysed by a microdiffusion assay with barbituric acid/pyridine as reagent (C).[41] Each experiment was either performed three times (A, B) or twice (C) in order to determine the mean value and standard deviations.

A: Ches buffer (472 μL, 0.25 mol L^{-1}) and ACCbs (28 μL, 1.5 mmol L^{-1}, 42 nmol or 42 μL, 1 mmol L^{-1}, 42 nmol) were added to a HGJ sample (V_{sample} = 0.5 mL). After one minute, the sample was passed through a Chromabond C18ec column. The column was washed with water (3 mL). Immobilized corrinoids were eluted with MeOH (0.4 mL). The eluate volume was adjusted to 0.5 mL, transferred to a quartz cuvette and analyzed with UV-vis. The concentration of cyanide in HGJ was determined with the help of the calibration curve shown in Fig. 4.

B: Ches buffer (200 μL, 0.25 mol L^{-1}) and ACCbs (42 μL, 1 mmol L^{-1}, 42 nmol) were diluted with water (758 μL, $V_{sample} = 1$ mL). Aliquots (10–100 μL) of diluted HGJ (dilution factor: 10) were added. After 5 minutes, the concentration of cyanide in HGJ was determined with the help of the calibration curve shown in Fig. 3 after volume correction and under considering of the dilution factor.

C: Sulfuric acid (10%, 0.5 mL) and diluted gastric juice (1 mL, 1 : 50 and 1 : 100 dilution with distilled water, respectively) were added to the outer compartment of two Conway microdiffusion cells. To the inner compartment sodium hydroxide (1 M, 0.5 mL) was added. The cells were immediately closed, gently agitated and incubated for 2 hours. An aliquot (0.1 mL) of the inner compartment was transferred into a tube containing a hydrogen phosphate solution (0.87 mol L^{-1}, 1 mL). A chloramine-T solution (25%, 0.5 mL) was added and incubated for 3 min at room temperature. Finally, a barbituric acid/pyridine reagent (1.5 mL; reagent: 3 g of barbituric acid in 15 mL of pyridine and hydrochloric acid (32%, 3 mL) were diluted with distilled water to a final volume of 25 mL) was added. It was incubated for another 10 min and the absorbance of the solution was measured at 580 nm. Concentrations were calculated by comparison with a calibration curve using standard cyanide solutions prepared in the same manner.

3. Results and discussion

Fig. 1 shows the UV-vis spectrum of aquacyanocobester (ACCbs; 42 μmol L^{-1})[36,40] in pure water as well as in a diluted sample of artificial gastric juice (AGJ; see experimental part).

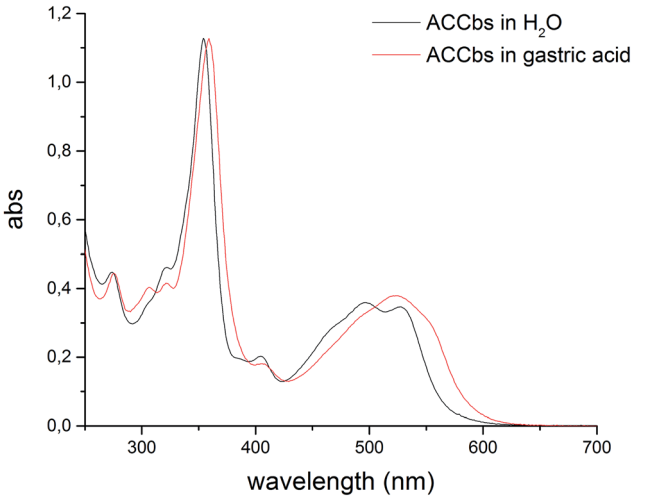

Fig. 1 UV-vis spectra of ACCbs (42 nmol) recorded in water and artificial gastric juice (AGJ : H_2O = 1 : 1; V = 1 mL).

The hypsochromic shift of the γ-band of ACCbs from 355 nm to 359 nm and the broadening of the α-, and β-bands (497 and 527 nm) to one single absorption (525 nm) indicates interactions between the chemosensor and AGJ. Most likely, an amino acid side chain of the pepsin, the main component of AGJ, is coordinated to the vacant coordination site of the chemosensor (Fig. 2A).[42,43]

Titrating cyanide ($[CN^-]$ = 0–160 μmol L^{-1}) to these solutions led to the stepwise formation of the dicyano-form of the indicator (Fig. 2 and 3). This behaviour is indicated by shifts of the γ-band from 359 nm to 368 nm and of the α/β-band from 526 nm to 583 nm. These characteristic absorptions are identical with those of dicyanocobester in water.[36,44]

Saturation of the chemosensor (42 μmol L^{-1}) with cyanide is reached in AGJ containing solutions at ~110 μmol L^{-1}. This concentration is approximately twice as high as for the same experiment in pure water (pH 7.5).[36] This difference can be rationalized by the competitive binding of pepsin to the chemosensor (Fig. 2A) that makes coordination of cyanide more challenging (Fig. 2C) compared to the reaction of cyanide with the 'free' chemosensor (Fig. 2B).

After having established the detection of cyanide in AGJ under homogenous conditions, we evaluated the possibility to combine the corrin-based chemosensors with solid phase extraction.[24,26,27,45] For this purpose, the reagent was added to a cyanide spiked AGJ sample and extracted afterward on the top of a commercially available reverse phase extraction column as described in the experimental section. The chemosensor is thereby trapped on the top of the C18ec column and is visible as a coloured ring. Depending on the concentration of cyanide, its colour varies between orange (aquacyano-form) and violet (dicyano-form).[27,36] For quantitative determinations, the reagent was subsequently eluted from the column with MeOH and analysed with UV-vis spectroscopy. The spectra unambiguously indicate a shift of the chemosensor ($\lambda_{\gamma\text{-band}}$ = 359 nm; $\lambda_{\alpha/\beta\text{-band}}$ = 526 nm) to its dicyano-form ($\lambda_{\gamma\text{-band}}$ = 368 nm; $\lambda_{\alpha\text{-band}}$ = 583 nm) with increasing concentrations of cyanide (Fig. 4 right). Simultaneously, strongly pronounced increases in absorbance are observed. For example, the intensity of the γ-band increases 2.4 times during titrations (Fig. 4 right), whereas it changes solely by ~20% in the same experiment under homogenous conditions (Fig. 3 left). This behaviour has been observed earlier and is explained by the more efficient extraction of the neutral dicyano-compound compared to the more polar positively charged aquacyano-form during SPE.[24] The corresponding calibration curve for cyanide shows a linear range up to 120 μmol L^{-1} (Fig. 4).

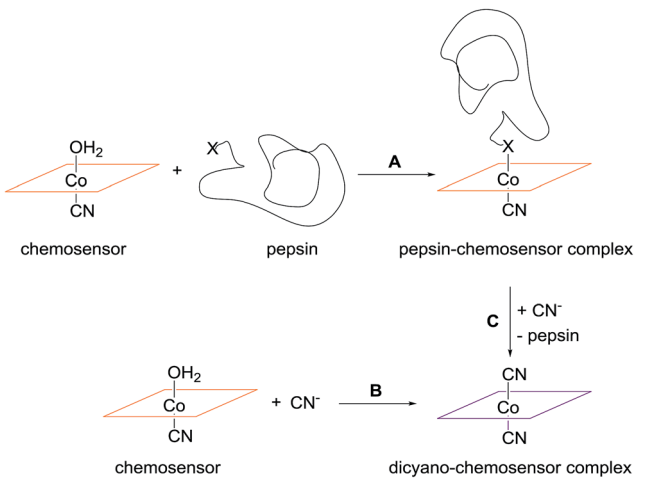

Fig. 2 Proposed coordination of pepsin (A) and cyanide (B) to the chemosensor. The displacement of pepsin with cyanide from the pepsin–chemosensor complex forming the violet coloured dicyano–chemosensor complex is shown in C (X: coordinating group).

Having demonstrated that cyanide detection is possible in AGJ with corrin-based chemosensors, we studied potential influences of incubation times and modes of sample storage.

Cyanide spiked samples of AGJ were stored at room temperature for up to 24 hours and analysed at different time intervals. No significant deviations were observed indicating that 'free' cyanide is sufficiently stable in AGJ under these conditions (Fig. 5). Storage at −21 °C was also tested (t = 24–1320 h). In this series of experiments, the detectable amount of cyanide dropped by 29 ± 5% after one week of storage (data not shown). Surprisingly, longer storage times did not show any additional negative effect and cyanide can be still detected in AGJ with corrin-based chemosensors and SPE after 55 days of storage at −21 °C.

After having established the corrin-based SPE method for cyanide detection in AGJ, the system was tested in a proof-of-concept forensic study. An anonymous sample from a suicide case, in which the victim had swallowed a solution of potassium cyanide, was evaluated. In contrast to the colorless AGJ sample, the human gastric juice sample (HGJ) was slightly reddish indicating the presence of additional, unknown components. Samples of HGJ were therefore first tested with SPE to avoid any undesired colour interferences from the HGJ sample.[27] Indeed, a clear color change of the chemosensor from orange to violet was observed while testing the HGJ samples (Fig. 6 left). This behaviour suggests the presence of free cyanide in the HGJ sample as supported by UV-vis spectroscopy after eluting the reagent from the column (Fig. 6 right). The absorbance spectrum of the eluted compound indicated unambiguously coordination of cyanide to the chemosensor generating its corresponding dicyano-form.[36] Quantification of cyanide in HGJ was then performed with the help of the previously established calibration curve (Fig. 4).

The analysis of a 267 times diluted HGJ sample indicated a cyanide concentration of 11 900 ± 900 μmol L^{-1}. This result is in very good agreement with an independent microdiffusion assay using barbituric acid/pyridine as reagent.[41] This assay showed a cyanide concentration of ~10 800 ± 100 μmol L^{-1} in HGJ of the suicide victim. Apart from this quantitative accordance, it is important to note that cyanide was still detected by 'naked-eye' in a ~1000 times diluted sample with corrin-based chemosensors and SPE.

In another set of experiments, we tested whether the extraction step of the chemosensor from the HGJ sample could be omitted and tried to directly determine the concentration of

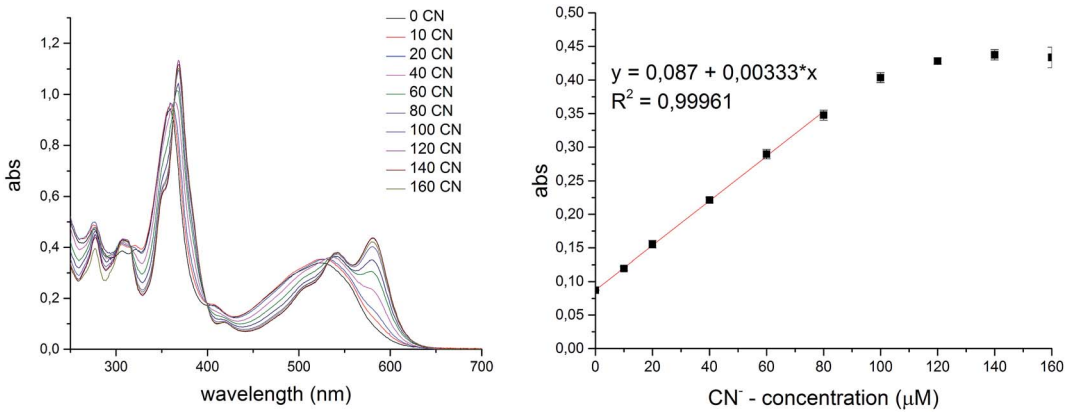

Fig. 3 UV-vis spectra of cyanide spiked AGJ (*left*) (AGJ : H_2O = 1 : 1; V = 1 mL; [CN^-] = 0–160 μmol L^{-1}, ACCbs = 42 nmol; pH 9.5) and the corresponding calibration curve (right).

cyanide in HGJ with the corrin-based reagent. However, although the formation of the corresponding dicyano-form of the chemosensor could by easily detected in the diluted HGJ sample (dilution factor: 969), quantification led to larger deviations compared to the SPE technique. For example, we calculated a cyanide concentration in HGJ of 16 100 ± 800 μmol L^{-1} instead of ~11 000 μmol L^{-1}. Apparently, the calibration curve generated from cyanide spiked AGJ (Fig. 3) cannot be used for this experimental set-up. Indeed, we demonstrated in this publication that the overestimation can be avoided by separating the reagent from the HGJ sample with SPE. This behaviour suggests the existence of additional (unknown) interferents in HGJ compared to AGJ that are removed during washings (see experimental part) with the SPE technique.

To the best of our knowledge, this technical note describes the unprecedented detection of cyanide in HGJ with chromogenic chemosensors. In particular, the results obtained with corrin-based chemosensor and SPE method are in very good agreement with the microdiffusion assay using barbituric acid/pyridine as reagent. However, the SPE method is advantageous in terms of sample preparation and speed of detection. In particular, only the conditioning of the column and sample dilution is required. Admittedly, only one authentic human sample was studied so far, but the results are encouraging for further studies in order to establish the system for routine

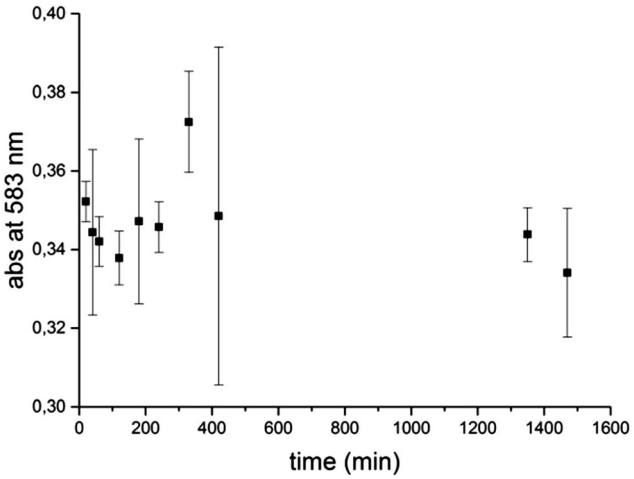

Fig. 5 Absorptions at 583 nm *versus* spiking times (t = 15–1470 min; V = 0.5 mL, [CN^-] = 40 μmol L^{-1}, ACCbs = 42 nmol; n = 3).

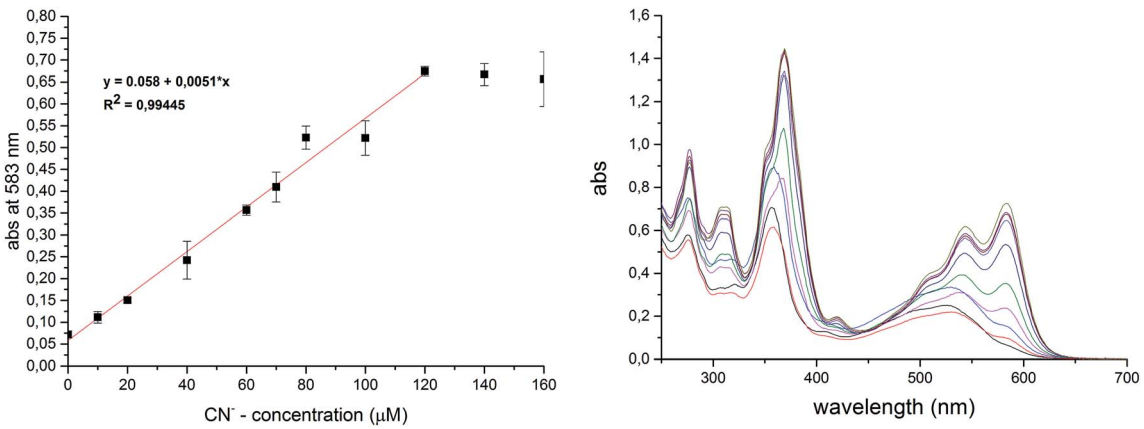

Fig. 4 Calibration curve of cyanide in AGJ (V = 0.5 mL) obtained by plotting the absorption at 583 nm *vs.* cyanide ([CN^-] = 0–160 μmol L^{-1}, ACCbs = 42 nmol; n = 3) (left). The corresponding UV-vis spectra are shown on the right.

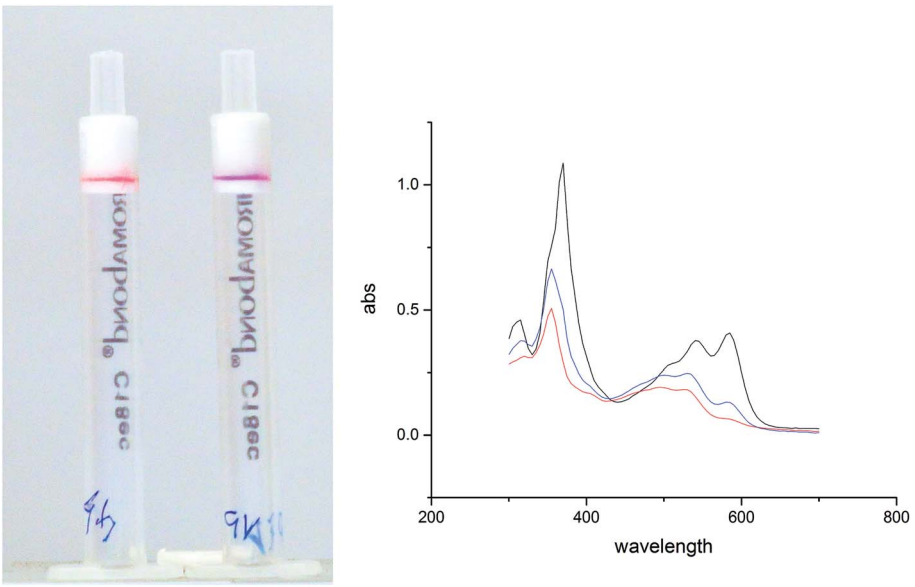

Fig. 6 Left: detection of cyanide in an HGJ sample using corrin-based chemosensors (ACCbs = 42 nmol) and SPE. Negative control (left column) and a diluted HGJ sample (dilution factor: 88; right column) right: UV-vis spectrum after elution of the chemosensor with MeOH from the solid support (orange: negative control, blue: dilution factor 1052; olive-green: dilution factor 88).

forensic investigations. These efforts would greatly benefit from the simultaneous rapid detection of cyanide in authentic human blood samples and corresponding forensic studies are planned for the future.

4. Conclusions

The detection of cyanide in artificial as well as authentic human gastric juice samples using corrin-based chemosensors and SPE extraction is described. The influence of spiking times and modes of storage were evaluated. It has been demonstrated that rapid, straightforward qualitative yes/no evaluations by 'naked eye' is possible for concentrations of cyanide in HGJ as low as 10 μmol L^{-1}. Quantitative measurements are in very good agreement with an independent microdiffusion assay using barbituric acid/pyridine as reagent. The SPE test-kit is therefore expected to complement existing current methods, especially when rapid qualitative and quantitative analyses of cyanide in HGJ are required.

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