METHOD FOR THE DETECTION OF TRICHLOROACETIC ACID IN URINE USING UV-VIS SPECTROPHOTOMETER

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INTRODUCTION

Trichloroacetic acid (TCAA) is excreted in urine as a metabolite of trichloroethylene (TCE) and perchloroethylene (PER) (EPA 2011). Both TCE and PER are volatile organic solvents widely applied as degreasers of fabricated metal parts, as lubricants, and in dry-cleaning. TCE is a well-known animal carcinogen, recognized for its many toxic effects in humans, such as cardiovascular effects, pulmonary toxicity, neurotoxicity and probably genotoxicity. Occupational exposure to TCE and PER occurs mainly through inhalation and less commonly through ingestion and dermal absorption.

Although TCAA levels cannot represent the severity of a TCE-induced disorder, it is helpful when linking health effects to TCE exposure; for example, dermatitis. The half-life of TCAA excretion in urine is about 2 to 5 days, therefore, urine for TCAA analysis should be collected at the end of working day shift of the week (Calafat., 2003).

Biological exposure indices; HSL (2013)

TCE=15mg/L (90 umol/L)

PER=7mg/L (42 umol/L)

Recently, Analytical Service has been receiving testing requests for TCE and PER. Samples have been sent to referral laboratories because there was no method available for such tests. Instead of outsourcing the test, the method was developed and validated so that samples can be analysed in-house.

METHODS USED FOR QUANTIFYING TCAA

Gas chromatography-MS

Headspace-GC FID

Spectrophotometry (Fujiwara colour reaction method)

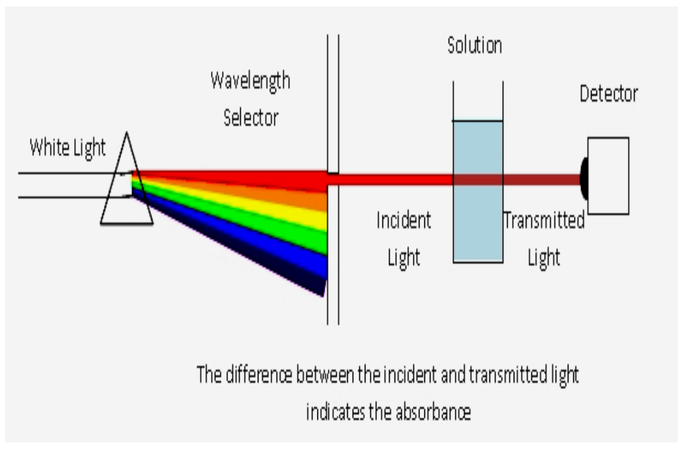


Figure 1: Spectrophotometer principle

Beer-Lambert Law.

Whenever a beam of monochromatic light is passed through a solution with an absorbing substance, the decreasing rate of the radiation intensity along with the thickness of the absorbing solution is actually proportional to the concentration of the solution and the incident radiation.

$A = \varepsilon lc$

AIM

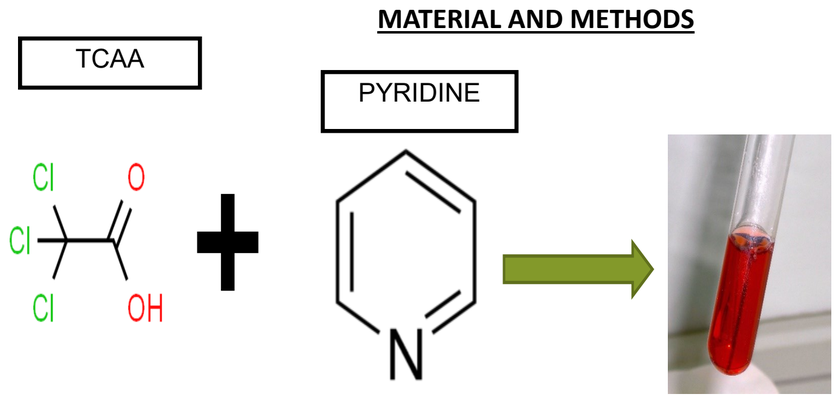
The aim of this study was to develop and validate a method for the routine analysis of TCAA in urine in the Analytical Service Department of the NIOH.

OBJECTIVES

To determine TCAA using UV-VIS-spectrophotometry.

To calculate the following figures of merit: uncertainty of measurement (UoM), linearity, accuracy as per precision and recovery, LOD and LOQ.

MATERIAL AND METHODS



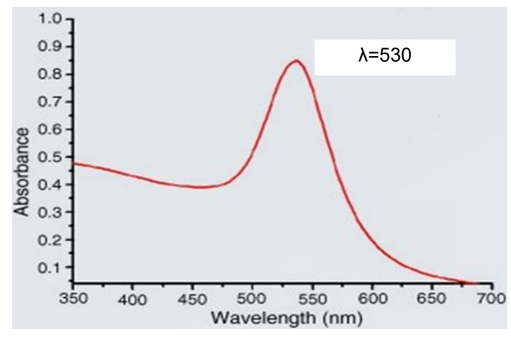


Figure 1: Absorption spectrum of TCAA at 530 nm

Urine of an unexposed individual was used to prepare calibration standards by adding varying amounts (0-150 umol/L, n = 15) of a known concentration of TCAA. Calibration standards and certified reference materials (CRMs) were prepared by adding pyridine and an alkaline solution of potassium hydroxide (7.8M). The mixture was heated at 65 ^{0}C for 30 min and produced a pink/purple colour. The samples were then cooled in an ice box for 15 mins. The pink/purple reaction product was determined photometrically at 530 nm using an Agilent Carry 60 UV-VIS Spectrophotometer.

Figure 2: AGILENT CARY 60 UV-VIS SPECTROPHOTOMETER

RESULTS AND DISCUSSION

Table 1: Analytical figures of merit for developed UV-VIS Spectrophometer method

Parameters	QC1	QC2
PRECISION (%CV)	13,58	9,14
RECOVERY (%)	90,32	95,45
LOD (µmol/L)	0,029	
LOQ (µmol/L)	0,097	
LINEARITY (r^2)	0.9994	
EXPANDED UoM (%)	11.29	

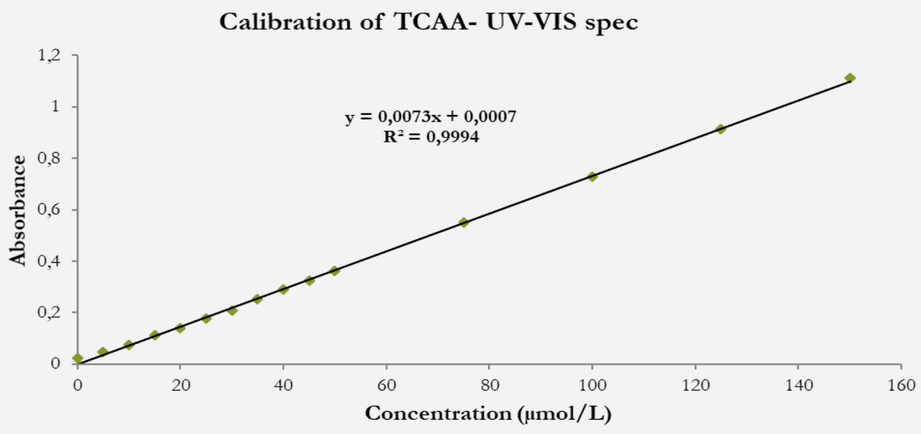


Table 2: Determination of TCAA by Head-space gas chromatography

PARAMETERS	RESULTS
Standard deviation	0,001-0,79
Recovery (%)	95,7-104,3
LOD (µmol/L)	0,002
Linearity (r)	0,999

Ohara et al.,(1991)

The method was validated and figures of merit calculated according to the current methods as stipulated in NIOH0391.

Linearity– the method was found to be linear in a range of 5 -150 µmol/L which covers the BEI for both TCE and PER. The calibration generated a correlation coefficient (r) of 0.9994. The linearity of the calibration curve confirms that the absorbance and concentration are proportional and obeys Beer's law.

LOD and LOQ– limit of detection and quantification were analysed using sample blanks (n=10) resulting in 0.029 and 0.097 respectively. The LOD and LOQ are both lower than the BEI which means the sensitivity of the method is good enough and hence the method is fit for purpose; that is, for the routine analysis of TCAA.

Method precision– The precision of the method is 13.58% (low concentration) and 9.14% (high concentration) which is acceptable according to NIOH0391.

Percentage recovery - The recovery of CRMs (QC1and QC2) ranged from 90-100% fulfilling the requirements of NIOH0391.

In a study conducted by Ohara et al., (1991) (Table 2) for the determination of TCAA in urine using head-space gas chromatography showed results with very low LOD of 0.002, good recovery of 95,7-104,3 %, excellent linearity with a correlation coefficient of 0.999 and standard deviation of 0.001-0.79. Though head-space gas chromatography is more sensitive than UV-VIS spectrophotometry, it is not ideal for routine analysis of TCAA but for research purposes, since it is time-consuming (approximately 6 minutes per sample), costly, impurities from the gas can easily interfere with the results and it needs intensive training to operate. On the other-hand the UV-VIS spectrophotometric method offers good results at a low cost, it is time efficient (approximately 20 samples in six minutes), and easy to operate with minimal risks. Therefore, this method is ideal for the routine analysis of TCAA in urine, for example in biological monitoring for occupational exposure to TCE.

CONCLUSIONS

The presented UV-VIS Spectrophotometric Method proved to be adequate for the routine analysis of TCAA in urine based on the calculated figures of merit, namely the linearity, percentage recovery, LOD and LOQ, precision and UoM. The method is to use, rapid and accurate and is therefore suitable and fit for purpose for biological monitoring for occupational exposure to TCE and PER.

REFERENCES

- Calafat AM, Kuklenyik Z, Caudill SP, Ashley DL [2003]. Urinary levels of trichloroacetic acid, a disinfection by-product in chlorinated drinking water in a human reference population. Environ Health Perspect 111(2):151-154.
- Kuklenyik Z, Ashley DL, Calafat AM [2002]. Quantitative detection of trichloroacetic acid in human urine using isotope dilution high-performance liquid chromatography-electrospray ionization tandem mass spectrometry. Anal Chem 74:2058-2063.
- M. S. Moss and H. J. Rylance: The Fujiwara reaction: some observations on the mechanism. Nature 210, 945–946 (1966).
- Mantel, M. and Nothmann, R., 1977. Rapid determination of trichloroethanol and trichloroacetic acid in urine. *Analyst, 102* (1218), pp.672-677.
- Ohara, A., Michitsuji, H., Jyoyama, Y., Yamaguchi, K. and Fujiki, Y., 1991. Determination of trichloroacetic acid and trichloroethanol by head-space gas chromatography (HS. GC). *Sangyo igaku. Japanese journal of industrial health*, *33*(2), pp.94-103.
- US EPA [2011]. Trichloroacetic acid (CASRN 76-03-9). United States Environmental Protection Agency. www.epa.gov/iris/subst/0655.htm (accessed April 7, 2014).