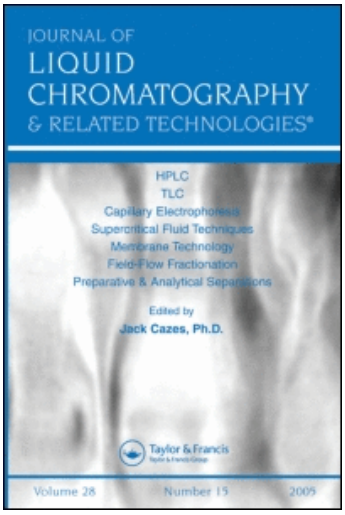

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

A Simple Method for the Quantification of Urethane in Spirits

Eugenie Jaryj[a]; Klemens Lorenz[a]; Bernd Spangenberg[a]
[a] University of Offenburg, Institute of Process Engineering, Offenburg, Germany

To cite this Article Jaryj, Eugenie , Lorenz, Klemens and Spangenberg, Bernd(2008) 'A Simple Method for the Quantification of Urethane in Spirits', Journal of Liquid Chromatography & Related Technologies, 31: 13, 1969 — 1976
To link to this Article: DOI: 10.1080/10826070802197370
URL: http://dx.doi.org/10.1080/10826070802197370

Journal of Liquid Chromatography & Related Technologies®, 31: 1969–1976, 2008

ISSN: 1082-6076 print/1520-572X online
DOI: 10.1080/10826070802197370

A Simple Method for the Quantification of Urethane in Spirits

Eugenie Jaryj, Klemens Lorenz, and Bernd Spangenberg

University of Offenburg, Institute of Process Engineering, Offenburg, Germany

Abstract: We present an improved quantification method for urethane found in spirits. The quantification is based on a derivatization reaction using cinnamaldehyde in combination with phosphoric acid. Measurements were carried out in the wavelength range from 445 to 460 nm using a diode-TLC device. An LED was used for illumination purposes. It emits very dense light at 365 nm. The quantification range of urethane is in the lower ng range. By applying 20 μL of sprits, the urethane quantification range is from 320 μg/L to 8.1 mg urethane per litre of spirit. The range of linearity covers nearly two magnitudes. The method is cheap, fast and reliable, and is able to monitor all European legislation limits without time-consuming sample pre-treatments.

Keywords: Derivatization, Food analysis, HPTLC, Quantification, Urethane, Wine

INTRODUCTION

Urethane (Ethyl Carbamate) is carcinogenic to mice and is assumed to be carcinogenic to man as well.[1] Urethane causes permanent damage to the immune and nervous system as well as the bone marrow. It is produced in wine and spirits when grape pips or plum-stones are damaged during the fermentation process. Hydrocyanic acid can be released if the liquid

Correspondence: Bernd Spangenberg, University of Offenburg, Institute of Process Engineering, Badstrasse 24, D-77652 Offenburg, Germany. E-mail: spangenberg@fh-offenburg.de

is left too long in contact with the mash and damaged pips or stones. Urethane is formed in combination with light and ethanol. There are legislation limits in Canada and Switzerland. The legislation limit in Switzerland is 1 mg urethane per litre of spirits. In Germany an urethane content of 400 μg/L is legal, but spirits may not be sold if the content is over 800 μg/L.

Commonly, urethane is quantified using TLC-[1,3–5] or GC-methods.[2,6–10] Urethane shows no light absorptions above 200 nm and must be stained when using a TLC method. For staining, (4-dimethylamino)cinnamaldehyde in ethanolic hydrochloric acid,[1] (4-dimethylamino)benzaldehyde in ethanolic hydrochloric acid,[3] and (4-dimethylamino)benzaldehyde in aqueous hydrochloric acid may be used after oxidation with potassium permanganate.[4]

The purpose of this work is to develop an inexpensive and reliable urethane quantification method in spirits that is sufficiently sensitive to meet the legislation limits without time-consuming pre-treatment procedure.

THEORY

In planar chromatography, light is used for detecting separated sample spots by illuminating the TLC-plate from above with light of known intensity (I_0). If the illuminating light shows higher intensity than the reflected light (J), a fraction of light must be absorbed by the sample (the analyte) and/or the TLC-layer. If a sample spot absorbs light, the reflected light intensity of this spot (J) is smaller than the illuminating light. The difference between these light intensities is absorbed by the sample. The definition of the total absorption coefficient a is:

$$I_{abs} = I_0 - J = aI_0 \tag{1}$$

Increasing sample amounts will induce a decreasing light reflection (J) by constant illuminating light intensity (I_0). Therefore, a transformation algorithm is needed which turns decreasing light intensities into increasing signal values. Ideally, there should be a linear relationship between the transformed measurement data and the analyte amount.

With the abbreviation:

$$R_0(\lambda) = \frac{J(\lambda)}{I_0(\lambda)}, \tag{2}$$

theoretical considerations lead to following equation for transformation purposes that show linearity between the transformed measurement data

(TMD) and the absorption coefficient.[11]

$$TMD(\lambda) = k\left(\frac{1}{R_0} - R_0\right) + (R_0 - 1) = \frac{a}{(1-a)} \tag{3}$$

k: backscattering factor ($k \geq 0$ and $k \leq 1$)
a: absorption coefficient

The value of the so called backscattering factor k is in the range between 0 and 1 and depends on the scattering quality of the stationary phase. In TLC, the Kubelka/Munk theory is often used for evaluation purposes. The Kubelka/Munk theory was first published in 1931 and is based on the assumption that half of the scattered flux is directed forward and half backwards.[12] The backscattering factor in the Kubelka/Munk–theory is $k = 1/2$ and the correct Kubelka/Munk–expression is rendered from expression (3) as:

$$TMD(\lambda, k = 1/2) = \frac{(1-R_0)^2}{2R_0} = \frac{a}{1-a} \tag{4}$$

For $k = 0$, no incident light is reflected to the plate top.[11,13] Light leaving the TLC-plate at the top must, therefore, be fluorescence light which can be set positive using Expression (5).

$$TMD(\lambda, k = 0) = (R_0 - 1) \tag{5}$$

In general, the fluorescent light is shifted to higher wavelengths in comparison to the absorbed light. That means that the fluorescence usually shows lower energy than the absorbed light. Using the fluorescence formula instantly reveals compounds at a TLC-track showing fluorescence. The benefit of fluorescence measurements is that fluorescence signals are increased by increasing illumination light and this offers lower quantification limits.

EXPERIMENTAL

Sample Preparation

Spirits can be applied in amounts of 1 to 20 μL directly on the TLC-plate. The water phase can be removed from the alcohol/urethane phase by saturation of the sample with Na_2CO_3. This is recommended if, e.g., a large amount of sugar in a liqueur makes the sample application of 20 μL impossible.[2]

Preparation of Standards and Application on HPTLC Plates

All the chemicals used were of analytical reagent grade. Urethane with a purity of ≥99% was purchased from Fluka, Switzerland. Cinnamaldehyde (purity ≥98%), acetone, methyl-t-buthyl ether, methanol, phosphoric acid, and sodium carbonate were purchased from Merck, Germany. Polyethylene glycol 600 is from Riedel-deHaën, Seelze, Germany. Silica gel K60 Lichrospere® (with a fluorescent dye) was used as the stationary phase. The plates were obtained from Merck, Germany.

Stock solutions were prepared by dissolving 4,000 mg of standard urethane in 25 mL of methanol. For calibration purposes, the stock solution was subsequently diluted with methanol in order to apply amounts of 1 to 20 μL.

Samples and standards were spotted, dash-like (7 mm), on an HPTLC silica gel Lichrospere® plate (10 × 10 cm, with fluorescent dye) using a Desaga AS 30 device. The plates were developed in a vertical developing chamber, without vapour saturation, to a distance of 65 mm from the starting point, using methyl-t-buthyl ether, methanol (7 + 3, V/V) as the mobile phase.

Plate Staining

Cinnamaldehyde (80 μL) were dissolved in 40 mL acetone. Then 2.4 mL concentrated orthophosphoric acid was added. The staining solution is only stable for two days at ambient temperature. For the reaction to a fluorescent dye, the plate is immersed in the staining solution for 2 seconds. The wet plate is placed in an oven at 130°C for 10 minutes. The urethane is converted to a blue fluorescent compound appearing on a white-purple background. The fluorescence can be enhanced by a factor of 2 if the plate is dipped for 4 sec into a solution of 10% polyethylene glycol 600 dissolved in methanol. A blue fluorescence appears on a white background. The urethane fluorescence is measured between 445 and 460 nm.

Apparatus

For direct spectrophotometry of HPTL-plates, a Tidas TLC 2010 system (J&M Aalen, Germany) was used. The reflection attachment was made of 75 identical optical fibres with a diameter of 100 μm each (produced by TransMIT-Centre for Fibre Optics and Industrial Laser Applications, Gießen, Germany), designed as triple row interface, arranged in three lines of 25 fibres each. Two rows of this interface transport light of different wavelengths from a deuterium lamp and a tungsten lamp or

from an LED to the HPTLC plate. The 25 fibres of the third row transport the reflected light back to the diode array detector. This triple row array shows a length of nearly 3 mm and a resolution on plate of better than 145 μm.[13] In this detecting mode, light sources and detector are both placed 450 μm above the surface of the HPTLC plate. A Tidas-system with a wavelengths resolution of 0.8 nm was used for detecting. For fluorescence evaluation an LED (model: Ledmod 365.1, produced by Omicron Laserage, Rodgau, Germany) was used instead of the deuterium lamp. Averaged densitograms were obtained in the emission wavelength range from 445 to 460 nm using a measurement time of 0.5 seconds per spectrum. The calculation of peak areas is done by use of home-made integration software.[14]

RESULTS AND DISCUSSION

The advantage of cinnamaldehyde in comparison with (4-dimethylamino)benzaldehyde or (4-dimethylamino)cinnamaldehyde is that cinnamaldehyde has no non-binding electrons. The reaction product with urethane, therefore, shows fluorescence. A fluorescence contour-plot of a urethane separation (162.52 ng) in the range from 400 to 700 nm is shown in Fig. 1. The plot reveals a maximum of fluorescence between

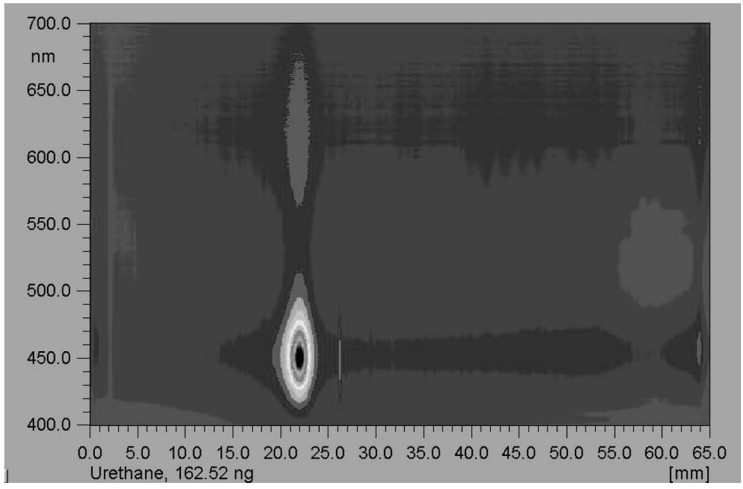

Figure 1. Plotted is the fluorescence contour-plot of a urethane standard separation (162.52 ng) on silica-gel, using methyl-t-buthyl ether, methanol (7 + 3, V/V) as the mobile phase. The urethane signal can be seen at 22 mm separation distance and the front signal is visible at 64 mm separation distance.

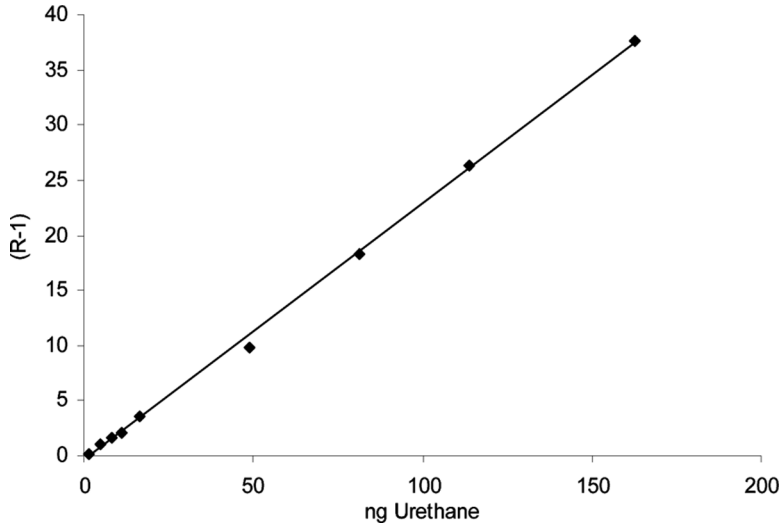

Figure 2. Plotted is the range of linearity for urethane from 1.6 ng to 162.5 ng.

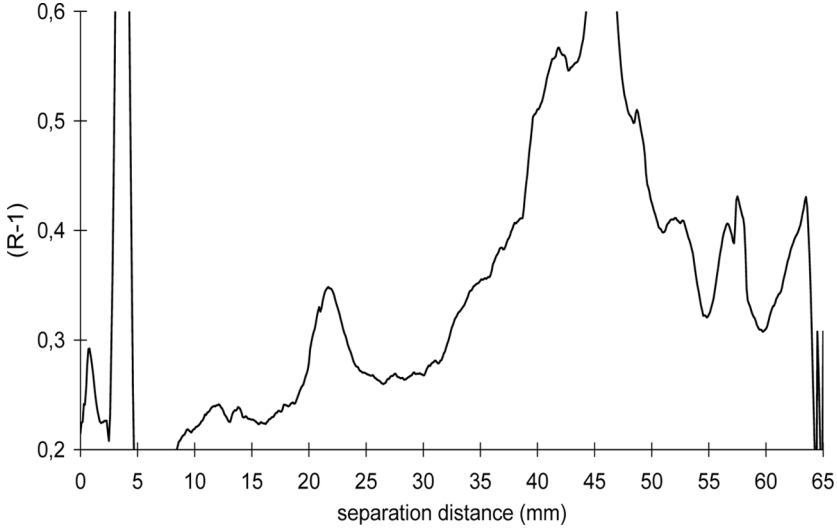

Figure 3. Plotted is the densitogram of a spirit sample, evaluated with expression (5) in the wavelength range from 445–460 nm. The spirit sample contains an amount of 0.7 mg/L urethane. The urethane signal can be seen at 22 mm separation distance and the front signal is visible at 64 mm separation distance.

445 and 460 nm. The calibration function of urethane shows linearity in the range from 1.6 to 162 ng. In Fig. 2, the calibration function of urethane is plotted.

The detection limit of the method, calculated as the triple peak-value of the baseline noise, is 4.8 ng for urethane. The quantification limit, calculated as 10-fold baseline noise peak-values, is 6.4 ng for urethane. The retardation factor of urethane is Rf = 0.31 if we use a new TLC-plate, just unwrapped from its packaging. The Rf-value for a TLC-plate, stored at 36% rel. humidity is Rf = 0.41. The standard deviation of four urethane samples at a 20 ng level (1 mg/L) is 7.1%.

A maximum of 20 μL spirits can be applied directly onto the HPTLC plate. If this is taken into consideration, the detection limit is 320 μg/L and the working range is up to 8.1 mg/L. The densitogram of a spirit sample (20 μL) containing 14 ng urethane (700 μg/L spirit) is plotted in Fig. 3.

The presented method works very reliably. Small changes in the mobile phase constitution do not alter the separation too much. The fluorescence on plate remains stable for weeks.

ACKNOWLEDGMENTS

The authors express their appreciation to J&M GmbH (Aalen, Germany), Merck-Company (Darmstadt, Germany) and DESAGA GmbH (Wiesloch, Germany) for support.

REFERENCES

1. Boyland, E.; Nery, R.J. The metabolism of urethane and related compounds. Biochemistry **1965**, *94*, 198–208.
2. Adam, L.; Bartels, W.; Christoph, N.; Stempfl, W. *Brennereianalytik. Qualitätskontrolle und Analytik im Fachlabor*; Band 2; Behr's Verlag: Hamburg.
3. Knappe, E.; Rohdewald, I. Dünnschichtchromatographie von substituierten Harnstoffen und einfachen Urethanen. Z. Anal. Chem. **1966**, *217*, 110–113.
4. Vassileva-Alexandrova, P.; Neicheva, A. Determination of the oxidative degradation products of ethylenethiourea by thin layer chromatography. J. Planar Chromatogr. **1996**, *9*, 217–220.
5. Fishbein, L.; Zielinski, W.L. Chromatography of carbamates. J Chromatogr. Rev. **1967**, *9*, 37–101.
6. Haase-Aschoff, K.; Haase-Aschoff, I. Untersuchung von Wein und Spirituosen auf Ethylcarbamat, Lebensmittelchem. Gerichtl. Chem. **1987**, *41*, 1–10.

7. Adam, L.; Postel, W. Gaschromatographische Bestimmung von Ethylcarbamat in Spirituosen. Branntweinwirtschaft **1987**, *127*, 66–67.
8. Andrey, D.Z. A simple gas chromatography method fort the determination of ethylcarbamate in spirits. Lebensm. Unters. Forsch. **1987**, *185*, 21–23.
9. Mildau, G.; Preuß, A.; Frank, W.; Heering, W. Ethylcarbamat (Urethan) in alkoholischen Getränken: Verbesserte Analyse und lichtabhängige Bildung. Dtsch. Lebensm. Rdsch. **1987**, *83*, 69–74.
10. Dennis, M.J.; Howarth, N.; Massey, R.C.; Parker, I.; Scotter, M.; Startin, J.R. Method for the analysis of ethylcarbamate in alcoholic beverages by capillary gas chromatography. J. Chromatogr. **1986**, *369*, 193–198.
11. Spangenberg, B. Does the Kubelka-Munk theory describe TLC evaluations correctly? J. Planar Chromatogr. **2006**, *19*, 332–341.
12. Kubelka, P.; Munk, F. Ein Beitrag zur Optik der Farbanstriche. Z. Tech. Phys. **1931**, *11a*, 593–601.
13. Spangenberg, B.; Klein, K.F. New evaluation algorithm in diode-array thin-layer chromatography. J. Planar Chromatogr. **2001**, *14*, 260–265.
14. Spangenberg, B. A new proposal for a parameter-free integration software. Fresenius J. Anal. Chem. **1998**, *360*, 148–151.

Received December 14, 2007
Accepted December 31, 2007
Manuscript 6311D