Fourier transform infrared and near-infrared spectroscopic methods for the detection of toxic Diethylene Glycol (DEG) contaminant in glycerin based cough syrup

M. Khalique Ahmed^{a,*}, Michael P. McLeod^b, Jean Nézivar^a and Allison W. Giuliani^c

^a *Department of Science, College of Liberal Education, Lynn University, North Military Trail, Boca Raton, FL, USA*

^b *University of Miami, Miller School of Medicine at Florida Atlantic University, Boca Raton, FL, USA*

^c *Department of Biological Sciences, Florida Atlantic University, Boca Raton, FL, USA*

Abstract. Recently there have been reports of the contamination of cough syrups with Diethylene Glycol (DEG). The consumption of such cough syrups has devastating effects on the health. In this paper we report evidence that Fourier transform infrared (FT-IR) and near-infrared (NIR) spectroscopic techniques are viable, simple, cost effective, rapid and fool proof methods for the identification and quantification of DEG in glycerin based cough syrups. The FT-IR and NIR spectra of the glycerin based cough syrup and up to 50:50 mixtures of DEG in cough syrup are recorded. The major peaks in the FT-IR spectrum of the cough syrup are assigned to the OH stretching ($\sim 3300\text{ cm}^{-1}$), CH stretching ($\sim 2900\text{ cm}^{-1}$), CH bending ($1500\text{--}1200\text{ cm}^{-1}$) and C–O stretching ($1200\text{--}900\text{ cm}^{-1}$) vibrational modes. In the FT-IR spectra of the mixtures, DEG contribute distinct peaks due to the vibrations of the C–O (920 cm^{-1}) and OC_2H_4 (892 cm^{-1}) moieties of its backbone and form the basis of the DEG detection and quantification. The prominent peaks of the NIR spectra of cough syrup and DEG are assigned to the first overtones of OH and CH, and to the combination of OH and CH fundamental vibrations. Both FT-IR and NIR Partial Least Square (PLS) calibrations produced correlation coefficients of 0.98.

Keywords: Infrared spectroscopy, near-infrared spectroscopy, cough syrup, diethylene glycol, contaminant, detection

1. Introduction

Diethylene Glycol (DEG) is an inexpensive, sweet tasting [1–3], industrial solvent commonly used in antifreeze solutions [1,4]. It is often substituted for more expensive chemicals [1,4] such as glycerin and propylene glycol [3,6] in pharmaceutical products such as tooth paste, injectable drugs, fever medication and cough syrup [8]. DEG has a molecular weight of 106.12 g/mol, a boiling point of 245°C , a melting point of -6.5°C and a vapor pressure of $<0.01\text{ mmHg}$ at 25°C [8]. It has been implicated in a number of serious world-wide contaminations with devastating human consequences; the first well documented case being the Massergil Tragedy of 1937 in the United States [5,6,9]. This tragedy was the stimulus behind passing the 1938 Federal Food, Drug and Cosmetic Act requiring product safety before being marketed [8,9]. Most recently in December 2008, Cable News Network (CNN) reported

*Corresponding author: M. Khalique Ahmed, Department of Science, College of Liberal Education, Lynn University, 3601 North Military Trail, Boca Raton, FL 33431, USA.

dozens of children deaths in Nigeria due to DEG poisoning. The symptoms of DEG poisoning include acute renal failure [1,3,5,6,10], metabolic acidosis [1,5,6], diarrhea [1,6], vomiting [1,3,6], and delayed (1–2 weeks post ingestion) neurotoxicity [4,9], i.e., optic neuritis, demyelination of peripheral and cranial nerves, and cerebral atrophy. The minimum toxic dose is not readily agreed upon; however, the median toxic dose for DEG has been reported to be 1.34 ml/kg with a range from 0.22–4.42 ml/kg [7]. DEG has been postulated to be toxic when converted by alcohol and aldehyde dehydrogenases to 2-hydroxyethoxyacetic acid (HEAA) [5,11]. DEG poisoning is best treated when discovered early [12–15] in the pathogenesis by implementing the alcohol dehydrogenase inhibitor fomepizole [3,5], ethanol infusion [2,15] and hemodialysis [3].

In the current study, we report evidence that FT-IR and NIR spectroscopic techniques are viable screening tools in the identification and quantification of DEG in glycerin based cough syrup.

2. Experimental section

Samples: Glycerin based Robitussin cough syrup was purchased from the local pharmacy. Active ingredient of the syrup was dextromethorphan HBr (15 mg/5.0 ml) and the major inactive ingredients were glycerin and water. The DEG was purchased from Sigma Aldrich and had the stated purity of 99%. The glycerin of 99% purity was purchased from the Fisher Scientific. The cough syrup was spiked with known amounts of DEG ranging from 3 to 50% DEG. The resultant samples were vortexed to perform infrared and near-infrared spectroscopic measurements.

FT-IR spectra: FT-IR spectra were recorded on a Bruker Vector 33 spectrometer equipped with a KBr beam splitter and deuterated triglycine sulfate (DTGS) room temperature detector. The spectra were recorded in the attenuated total reflection (ATR) mode with a single bounce MIRacle® ATR accessory from PIKE technologies, Inc. (Madison, WI, USA). The accessory was equipped with a split pea shaped ZnSe ATR crystal. To record spectra, about 10 μ l of sample solution was poured on the ATR crystal and the single beam spectrum was recorded and then was rationed against the single beam spectrum of the bare crystal to obtain the absorbance spectrum. Spectra were recorded with a resolution of 4 cm^{-1} and 64 scans were averaged for each spectrum. The spectrometer's optics were sealed from the atmosphere but its compartment was not purged during measurements.

NIR spectra: The near-infrared spectra were recorded in the absorbance mode using 1 mm rectangular quartz cell. The instrument (Bruker Vector 33) was equipped with the tungsten source, CaF_2 beam splitter and the germanium detector. The spectra were run at the resolution of 4 cm^{-1} and for each spectrum 64 scans were averaged.

Data processing: The spectra were run and processed with the Bruker OPUS (Optical User Software) program. The recorded infrared and near-infrared spectra were subjected to the Partial Least Squares (PLS) analysis using QUANT program of the Bruker Instruments. The procedure produced the actual and predicted values of DEG in cough syrup.

3. Results and discussion

FT-IR spectra: The infrared spectra of glycerin based cough syrup and of DEG are shown in Fig. 1. The spectrum of cough syrup is dominated by peaks due to water and glycerin. The band at 3300 cm^{-1} is due to OH stretching modes of water and glycerin whereas the band at 1650 cm^{-1} is mainly due to OH bending mode of water. The other peaks of the cough syrup are mainly due to the presence of glycerin. The peaks around 2900 cm^{-1} are due to the CH stretching modes of glycerin whereas the peaks between

Fig. 1. Infrared spectra of glycerin based cough syrup and diethylene glycol. (The colors are visible in the online version of the article; <http://dx.doi.org/10.3233/SPE-2010-0482>.)

Fig. 2. Infrared spectrum of glycerin.

1500–700 cm^{-1} are due to CH bending (1500–1200 cm^{-1}) and C–O stretching (1200–900 cm^{-1}) modes of glycerin. The glycerin peaks in cough syrup were confirmed by running the spectrum of pure glycerin that is shown in Fig. 2. The assignments of the FT-IR spectra of the cough syrup, glycerin and DEG are given in Table 1. The closer inspection of the spectra of Fig. 1 revealed absorptions in the region of 930–870 cm^{-1} in the DEG spectrum that are absent in the cough syrup spectrum. These absorptions are possibly from the C–O (920 cm^{-1}) and OC_2H_4 (892 cm^{-1}) moieties of the backbone of DEG. This assignment is reasonable as glycerin does not have OC_2H_4 moiety and that is why 892 cm^{-1} absorption is absent in the cough syrup. The 930–870 cm^{-1} region peaks of DEG were used for the quantization of DEG in cough syrup through Partial Least Squares (PLS) procedure. Figure 3 shows the sensitivity

Table 1

Assignments of the infrared spectra of the cough syrup, Diethylene Glycol (DEG) and glycerin. Peak positions are in wavenumbers (cm^{-1})

Cough syrup	DEG	Glycerin	Assignment
3306	3340	3295	OH stretch
2938	2924	2932	CH ₂ antisym. stretch
2890	2870	2879	CH ₂ sym. stretch
1639	...	...	OH bending
1420	1455, 1419	1412	CH ₂ bending
1356	1362, 1324	1326	CH ₂ wagging
1150	1234	1209	CCC, COC stretch
1104	1125	1108	C–O stretch
1077	1062	1030	C–O stretch
1028	920	993, 922	COH stretch
...	892	...	–OC ₂ H ₄

Fig. 3. The infrared spectra showing the sensitivity of the contaminated cough syrup to the presence of varying amounts (0–50%) of diethylene glycol. (The colors are visible in the online version of the article; <http://dx.doi.org/10.3233/SPE-2010-0482>.)

of the FT-IR spectra of the contaminated cough syrups to the presence of varying amounts of DEG. As can be seen the $930\text{--}870\text{ cm}^{-1}$ peaks grow linearly with the concentration of DEG in cough syrup. The plot of PLS predicted and actual values of DEG from FT-IR spectra is shown in Fig. 4. The agreement between predicted and actual values is excellent.

NIR spectra: The near-infrared spectra of DEG and the cough syrup are shown in Fig. 5. In both spectra the bands between $5500\text{--}6000$ and $6500\text{--}7000\text{ cm}^{-1}$ regions are due to first overtone of CH

Fig. 4. Plot of the actual and predicted values of diethylene glycol in cough syrup from infrared spectroscopy.

Fig. 5. Near-infrared spectra of a typical glycerin based cough syrup and diethylene glycol. (The colors are visible in the online version of the article; <http://dx.doi.org/10.3233/SPE-2010-0482>.)

and OH stretching vibrations, respectively. As can be seen in Fig. 5 DEG has distinct absorptions in the regions of 6000–5500 cm^{-1} whereas cough syrup absorbs very weakly in this region. The assignments of the NIR spectra of the cough syrup and DEG are given in Table 2. Figure 6 displays the sensitivity of the near-infrared spectra of the cough syrup to the presence of DEG. Because of the mutually exclusive

Table 2

Assignments of the near-infrared spectra of the cough syrup and diethylene glycol

Frequency (cm^{-1})	Assignment
Cough syrup	
5630	First CH (glycerin) overtone
~ 6350	CH stretch + OH stretch
6855	First OH overtone
Diethylene glycol	
~ 5625	First CH overtone
5777	First CH overtone
6347	CH stretch + OH stretch
6772	First OH overtone
8244	Second CH overtone

Fig. 6. Near-infrared spectra showing the sensitivity of the contaminated cough syrup to the presence of varying amounts (5–50%) of diethylene glycol. (Colors are visible in the online version of the article; <http://dx.doi.org/10.3233/SPE-2010-0482>.)

Fig. 7. Plot of the actual and predicted values of diethylene glycol in cough syrup from near-infrared spectroscopy. (Colors are visible in the online version of the article; <http://dx.doi.org/10.3233/SPE-2010-0482>.)

nature of the $6000\text{--}5500\text{ cm}^{-1}$ spectral range, this region was used in the determination of the DEG in the cough syrup.

The predicted and actual values of DEG from the near-infrared spectra are shown in Fig. 7 and display reasonable agreement.

4. Conclusion

The presence of toxic diethylene glycol (DEG) can be successfully detected and quantified in the glycerin based cough syrup by using FT-IR and NIR spectroscopic techniques. It should be noted that although FTIR and NIR techniques are not as sensitive as other analytical techniques such as mass spectrometry and high pressure liquid chromatography (15) but they have the advantage of being rapid and cost effective. Both FT-IR and NIR are well suited for the initial screening of the cough syrups before using the most sensitive analytical techniques. The FT-IR and NIR methods are environmentally friendly as they do not utilize any chemicals. The developed methods are especially suited for the third world where advanced lab facilities are uncommon and are the regions where most cases of the cough syrup contaminations have been reported.

References

- [1] S. Alfred, P. Coleman, D. Harris, T. Wigmore, E. Stachowski and A. Gaudins, Delayed neurologic sequelae resulting from epidemic diethylene glycol poisoning, *Clinical Toxicology* **43**(3) (2005), 155–159.
- [2] B.D. Barr, J.R. Barr, G. Weerasekera, J. Wamsley, S.R. Kalb, A. Sjodin et al., Identification and quantification of diethylene glycol in pharmaceuticals implicated in poisoning epidemics: an historical laboratory perspective, *Journal of Analytical Toxicology* **31** (2007), 295–303.

- [3] L.A. Ferrari and L. Giannuzzi, Clinical parameters, postmortem analysis and estimation of lethal dose in victims of a massive intoxication with diethylene glycol, *Forensic Science International* **153** (2005), 45–51.
- [4] M.T. Gomes, M.I. Veríssimo and J.A. Oliveira, A new method for monitoring the contamination of glycerol with diethylene glycol, *Journal of Pharmacy and Pharmacology* **51** (1999), 233–236.
- [5] P. Hari, Y. Jain and S.K. Kabra, Fatal encephalopathy and renal failure caused by diethylene glycol poisoning, *Journal of Tropical Pediatrics* **52**(6) (2006), 442–444.
- [6] M.J. Hasbani, L.H. Sansing, J. Perrone, A.K. Asbury and S.J. Bird, Encephalopathy and peripheral neuropathy following diethylene glycol ingestion, *Neurology* **64** (1992), 1273–1275.
- [7] F. Hernandez, M. Ibanez and J.V. Sancho, Fast determination of toxic diethylene glycol in toothpaste by ultra-performance liquid chromatography – time of flight mass spectrometry, *Analytical and Bioanalytical Chemistry* **391** (2008), 1021–1027.
- [8] J. Marraffa, M. Holland, C. Stork, C. Hoy and M. Hodgman, Diethylene glycol: widely used solvent presents serious poisoning potential, *Journal of Emergency Medicine* **35** (2007), 401–406.
- [9] H. Maurer and C. Kessler, Identification and quantification of ethylene glycol and diethylene glycol in plasma using gas chromatography-mass spectrometry, *Archives of Toxicology* **62** (1988), 66–69.
- [10] H. Maurer, F.T. Peters, L.D. Paul and T. Kraemer, Validated gas chromatographic – mass spectrometric assay for determination of the antifreezes ethylene glycol and diethylene glycol in human plasma after microwave-assisted pivalylation, *Journal of Chromatography B – Biomedical Sciences and Applications* **754** (2001), 401–409.
- [11] K.L. O'Brien, J.D. Selanikio, C. Hecdivert, M.F. Placide, M. Louis, D.B. Barr et al., Epidemic of pediatric deaths from acute renal failure caused by diethylene glycol poisoning, *JAMA* **279** (1988), 1175–1180.
- [12] H.O. Okuonghae, I.S. Ighogboja, J.O. Lawson and E.J. Nwana, Diethylene glycol poisoning in Nigerian children, *Annals of Tropical Paediatrics* **12** (1992), 235–238.
- [13] L. Schep, R. Slaughter, W. Temple and D. Beasley, Diethylene glycol poisoning, *Clinical Toxicology* **47**(6) (2009), 525–535.
- [14] J.G. Schier, C.S. Rubin, D.M. Miller, D. Barr and M.A. McGeehin, Medication-associated diethylene glycol mass poisoning: a review and discussion on the origin of contamination, *Journal of Public Health* **30** (2009), 127–143.
- [15] J. Shin, G. Sachs and J. Kraut, Simple diagnostic tests to detect toxic alcohol intoxications, *Translational Research* **152** (2008), 194–201.