

Detection of Adulterants in Raw Materials, Drug Substances, and Products by NMR Spectroscopy

Charlotte A. Carroll, Michele L. DeRider, and Thomas N. Feinberg

Purpose

The contamination of drug products with economically-motivated adulterants (EMAs) has been a growing concern. These adulterants may be added to the raw materials or the drug substance at any stage in the preparation of the finished product. Tests for the purity of these materials have been proven to be too specific, and therefore, too easily deceived. Therefore, we propose nuclear magnetic resonance (NMR) spectroscopy as a general test for the presence of adulterants. The examples provided are of materials that have recently been found to have been intentionally contaminated. However, the method is general enough to be used to screen all materials throughout the process and to provide reasonable assurance as to the quality of those materials.



Methods

A series of samples were prepared to study the use of NMR spectroscopy to detect the presence of adulterants in raw materials (milk), excipients (glycerin), API (heparin), and finished drug product (acetaminophen syrup).

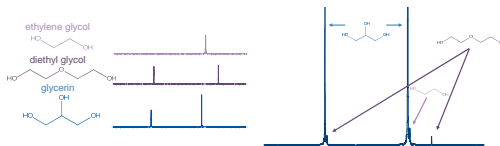
All NMR spectra were collected using a 500 MHz Varian UNITY INOVA NMR spectrometer equipped with a 5-mm switchable probe. Samples were analyzed by ^1H or ^{13}C . When required for ^1H spectra, water suppression, using either presaturation, and carbon decoupling was applied. The ^{13}C NMR spectra were collected with proton decoupling.



Diethylene Glycol and Ethylene Glycol in Glycerin

In 1937, more than 100 people were poisoned by diethylene glycol (DEG) used as an excipient in a sulfanilamide preparation. The Congressional response to the public outrage this disaster sparked was to pass the 1938 Federal Food, Drug, and Cosmetic Act.

Glycerin is a common excipient in many different products. Many more incidents of DEG poisoning from contaminated glycerin have occurred throughout the years in syrups and tooth paste. The USP Glycerin Monograph was revised to include a limit for DEG in the Identification test using gas chromatography. This example was chosen to demonstrate how even minor changes a compound can be detected using NMR spectroscopy.

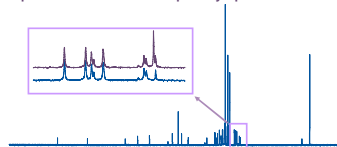


Experimental

Natural abundance carbon ^{13}C spectra of each separate component: glycerin, diethyl glycol and ethylene glycol in deuterated DMSO were collected. A mixture of 1% (w/w) of diethyl glycol and ethylene glycol in glycerin in DMSO was also analyzed. The NMR spectrum of the mixture was collected in approximately six minutes.

A sample of adult acetaminophen syrup was added directly to an NMR tube. A ^{13}C spectrum was collected without lock solvent. A drop of DEG was added approximately 1 mL of acetaminophen syrup and another ^{13}C spectrum was collected.

^{13}C spectrum of neat acetaminophen syrup
 ^{13}C spectrum of neat acetaminophen syrup + DEG



Results

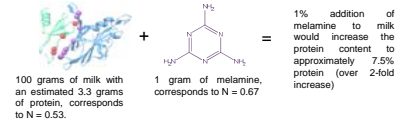
Ethylene glycol and diethylene glycol were easily detected in glycerin using ^{13}C detection. Even in the complex spectrum of neat acetaminophen syrup, a single drop of DEG in approximately one milliliter of neat syrup could be identified. In addition to allowing the detection of any other proton- or carbon-containing adulterants, this method is also faster than the published USP method at 14 minutes per analysis, instead of 23 minutes for the GC method.

Melamine and Cyanuric acid

Melamine contamination made the headlines in the United States in 2007 when a number of companion pets became ill and died. Even more tragic is the loss of at least six infants in China in 2008.

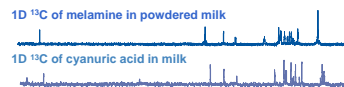
Protein content in foods is often determined in other countries using a non-specific quantification method called the Kjeldahl method. This method measures nitrogen levels, relying on the assumption that the amount of nitrogen present in foods is due from contribution from protein. Melamine can be added to fraudulently inflate the nitrogen content. Every molecule of melamine contains six atoms of nitrogen, so adding a small amount of melamine to milk would significantly increase the amount of nitrogen detected in the test. The melamine used for this purpose also often contains cyanuric acid, an analog that complexes with melamine to form crystals that accumulate in the kidneys.

Protein content calculated by multiplying the amount of nitrogen determined (N) by factor based on the typical nitrogen content in a protein (16% a common estimate). Milk is typically 3.3% total protein. Melamine contains 66.7% nitrogen by weight. Small amounts of melamine added greatly increases the calculated protein content using non-specific methods.



Experimental

A sample containing 1% (w/w) of melamine and powdered milk was dissolved in deuterated DMSO. The resulting slurry was centrifuged. The supernatant was placed directly in an NMR tube. Natural abundance carbon ^{13}C spectra of melamine in DMSO at a concentration of 1% (w/w) relative to powdered milk. Cyanuric acid solution in milk was also analyzed using ^{13}C NMR spectroscopy.



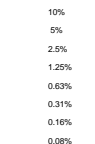
Results

Melamine and cyanuric acid were detected in a milk sample using ^{13}C detection. The use of NMR allows a single mode of analysis for both materials, instead of the two modes required for the LC-MS/MS method. Samples were analyzed without extraction or filtration, greatly simplifying the preparation.

Heparin

The U.S. Pharmacopeial (USP) Convention revised the monographs for heparin sodium and heparin calcium on June 18th, 2008. Stage 2 of those revision became official October 1st, 2009. The revisions were in direct response to the recent heparin crisis. FDA investigators found batches of heparin products containing a contaminant, later identified as oversulfated chondroitin. In the previous revision of the USP heparin monograph, the oversulfated chondroitin could appear to be heparin in some of the tests since it can mimic the anticoagulant properties of heparin. Both revisions of the heparin monograph utilize Nuclear Magnetic Resonance (NMR) spectroscopy to detect the presence oversulfated chondroitin in heparin drug products. The revised monographs test heparin sodium and calcium drug substances to assure their quality. All drug manufacturers who market heparin in the United States are required to meet these revised standards.

Weight % OSCS



Experimental

A series of heparin samples containing varying amounts of OSCS were prepared in deuterium oxide, reported in Weight % relative to heparin. The ^{13}C NMR spectra were collected with carbon decoupling with residual solvent signal presaturation.

Results

Oversulfated chondroitin sulfate was observed in heparin samples using ^{13}C detection as per current USP heparin sodium monograph. This example highlights the ability of a simple ^{13}C NMR method to screen for EMAs.

Conclusions

NMR spectroscopy has been proven to be an acceptable test for common EMAs in raw materials as well as in API. In addition, the method is general enough to be used to screen all materials throughout the process and to provide reasonable assurance as to the quality of those materials.