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science on demand

Hypromellose and Other Cellulose Type Assays by Gas Chromatography

Figure 1: Preparing to manually inject the standard solution

Butterworth Laboratories Ltd (BLL) routinely carries out the assay for hypromellose, methylcellulose, ethylcellulose, hydroxypropylcellulose and its low substitution counterpart to the USP, PhEur, JP and ChP for several clients. Due to the hazardous nature of the test, particularly the heating of hydriodic acid to temperatures up to 165°C, it is a commonly outsourced test within the pharmaceutical and other industries. As such, it has given the staff at BLL considerable experience and expertise in the successful execution of the test in its current form in the compendia as a gas chromatography (GC) assay but also in its previous wet chemistry / gravimetric technique.

The current GC assay can source its origins to the Zeisel Procedure named after the Czech chemist, Simon Zeisel. The test provided a simple means of determining alkoxy substituents in carbohydrates in their original form via a stoichiometric gravimetric test. The test involved adding excess hydriodic acid to cleave the alkoxy groups from the carbohydrate and convert them into the corresponding alkyl iodide. This was then passed over silver nitrate to form silver iodide, and this product was used to determine the content of alkoxy groups gravimetrically.

Today's compendial hypromellose GC assay (aforementioned sample types proceed similarly) involves using an internal standard in the form of n-octane in o-xylene. The sample is weighed into a reaction vial along with the catalyst: adipic acid. Internal standard and hydriodic acid (in excess) are added and the sample vial is then heated at 130°C to complete the cleavage of the methoxy and hydroxypropoxy groups and their subsequent conversion into methyl iodide and isopropyl iodide. These resulting analytes are then quantified by GC via manual injection (Figure 1) using the internal standard method against a standard of known concentrations of methyl and isopropyl iodide.

Prior to 2019, the USP, Ph. Eur. and JP prescribed using a packed column for the assay using a 3 to 4mm × 1.8 to 3m glass, packed with 20% liquid phase G1 on 100 to 120-mesh support S1. Since then, the pharmacopoeia (USP, PhEur and JP) have updated and harmonised their procedure using a 30m x 0.53mm x 3.0µm capillary column of G1 phase, or G43 with the ChP. Note: Hypromellose acetate succinate to the JP still uses a packed column. As expected, the peaks using the capillary column are much narrower, sharper and better resolved. See the two chromatograms below (Figures 2 and 3).

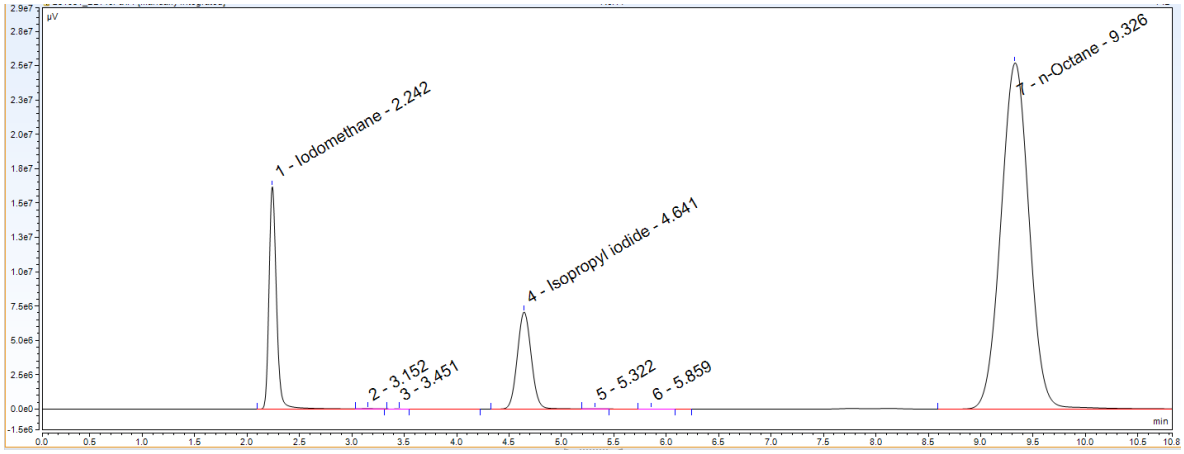

Figure 2. Hypromellose Standard Chromatogram (Packed column) USP

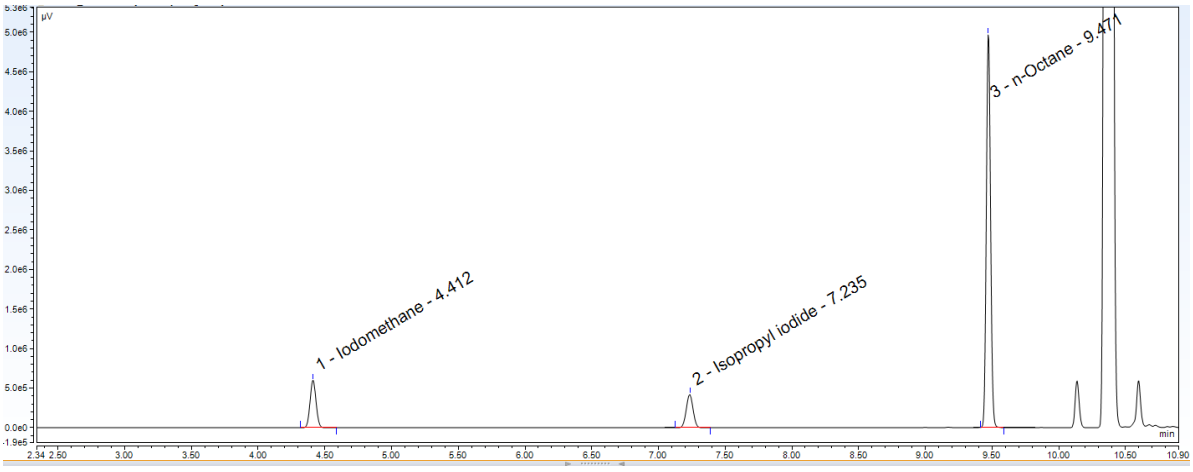

Figure 3. Hypromellose Standard Chromatogram (Capillary column) USP

Despite the improved chromatography obtained with the capillary column, the hypromellose assay is one of the few tests where the packed column method produces better results. This is particularly apparent pertaining to the repeatability and %RSD of the internal standard ratio with respect to methyl iodide. For the twenty-plus years of BLL performing the hypromellose assay with the packed column, there were no precision failures. However, higher RSD values of up to 2.0% became more frequent once the pharmacopoeia introduced the capillary column. Precision failures started to occur sporadically, but only for methyl iodide and not isopropyl iodide. Furthermore, with a limit of NMT 2.0% RSD and a specification of 28.0 – 30.0% methoxy groups for Hypromellose, a %RSD of 2.0% (n=6) could result in a sample manufactured at the lower or upper end of the specification to produce an out

of specification (OOS) result. The likely cause of the reduced precision with the introduction of the capillary column method will be the split. Due to the large variation in boiling points of methyl iodide (42°C) and the internal standard, o-xylene (144°C), split discrimination can be significant, which has been evidenced by the variations seen in the area ratios of methyl iodide to internal standard resulting in increased %RSD. More often than not, the %RSD of methyl iodide is twice that of isopropyl iodide, which has a higher boiling point of 89°, thereby keeping split discrimination of this component to a minimum.

To minimise the risk of false OOS results and improve on the data generated, BLL has implemented a more stringent %RSD of NMT 1.0% for Hypromellose assays to give our clients greater confidence in our performing this test. Since introducing this requirement, no quality events have been generated with the assay using capillary columns. Additionally, BLL's GC replacement program with Shimadzu GC-2030 gas chromatographs has further improved the precision, contributing to our continued confidence in producing good, accurate, consistent and reliable data.

Author Biography

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Andrew started his career at Siliker as an Analyst in 2002 before moving to Sigma Pharmaceuticals in Australia for four years in 2003. After a short spell with GSK in Beckenham, where he first started working on GC analysis, he joined Butterworth from 2008 to 2014 as an Analyst in the Chromatography Department. Spells at ESG and Broughton Laboratories followed this before returning as a Senior Analytical Chemist in 2020.