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Analysis of ethylene glycol and 1,4-butanediol by HPLC-RID

Forked from Analysis of sugars, small organic acids, and alcohols by HPLC-RID

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We use this protocol and it's working

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Disclaimer

This protocol is for research purposes only.

Abstract

An analytical method was developed using high performance liquid chromatography with refractive index detection (HPLC-RID) to quantify the concentration of ethylene glycol and 1,4-butanediol in aqueous samples. This list is non-exhaustive as this isocratic method with an acid modified mobile phase is compatible with analysis of many alcohols, small acids, and sugars. This method utilizes a Bio-Rad Aminex HPX-87H Ion Exclusion Column to provide chromatographic separation.

Guidelines

This protocol utilizes an high pressure liquid chromatography refractive index detector (HPLC-RID) system manufactured by Agilent Technologies as referenced in '*Materials*'. A similar HPLC-RID system can be utilized; however, some parameter nomenclature may deviate depending on the manufacturer.

Materials

Reagents:

10N Sulfuric Acid **Fisher Scientific** **Catalog #**SA200-1

Standards:

Ethylene glycol **Toronto Research Chemicals Inc** **Catalog #**E890140-1mL

1,4-butanediol **Merck MilliporeSigma (Sigma-Aldrich)** **Catalog #**493732-1L

Materials:

Syringe filters for aqueous matrices

Equipment	
syringe filters, 13mm nylon membrane	NAME
syringe filter	TYPE
Cytiva	BRAND
4550	SKU
https://www.cytivalifesciences.com/en/us/shop/lab-filtration/syringe-filters-non-sterile/nylon-non-sterile-syringe-filters/acrodisc-syringe-filters-with-nylon-membrane-p-36371	LINK
13mm	SPECIFICATIONS

Syringe filters for organic matrices-

Equipment

syringe filters, 13mm nylon membrane	NAME
syringe filter	TYPE
Cytiva	BRAND
4550	SKU
https://www.cytivalifesciences.com/en/us/shop/lab-filtration/syringe-filters-non-sterile/nylon-non-sterile-syringe-filters/acrodisc-syringe-filters-with-nylon-membrane-p-36371	LINK
13mm	SPECIFICATIONS

Instrumentation:

Equipment

Agilent 1260 Infinity II LC System	NAME
HPLC System	TYPE
Agilent	BRAND
Agilent 1260 Infinity II LC System	SKU
https://www.agilent.com/en/product/liquid-chromatography/hplc-systems/analytical-hplc-systems/1260-infinity-ii-lc-system	LINK
G7111B Quat Pump G7167A Multisampler G7116A 1260 MCT G7117C 1260 DAD HS G7162A 1260 RID	SPECIFICATIONS

Column:

Analytical Column

Equipment

HPX-87H Column

NAME

Column

TYPE

Bio-Rad

BRAND

125-0140

SKU

https://www.bio-rad.com/en-us/sku/1250140-aminex-hpx-87h-column?ID=1250140&WT.mc_id=170208017160&WT.srch=1&WT.knsh_id=_kenshoo_clickid_&gclid=EAIaIQobChMI5vbXvujS9wIVTxPUAR2GPgz2EAAYAiAAEgIuV_D_BwE

LINK

Guard Column

Equipment

Micro-Guard Cation H Cartridge

NAME

Guard Column

TYPE

Bio-Rad

BRAND

1250129

SKU

https://www.bio-rad.com/en-us/sku/1250129-micro-guard-cation-h-refill-cartridges?ID=1250129

LINK

Troubleshooting

Safety warnings

All chemicals used for this procedure are hazardous. Read the Safety Data Sheet (SDS) for all chemicals and follow all applicable chemical handling and waste disposal procedures. Manufacturer specific SDS information can be found by following the CAS numbers of compounds in 'Materials' list.

Sulfuric acid can cause serious chemical burns. See SDS for additional information:

https://beta-static.fishersci.com/content/dam/fishersci/en_US/documents/programs/education/regulatory-documents/sds/chemicals/chemicals-s/S25899.pdf

Before start

All solvents and chemicals used are listed in the ‘Materials’ section. These are excluded from in-line references to maintain clarity and keep the steps concise.

Preparation of mobile phase and instrument equilibration

1 Mobile phases

1. To make 0.01 N sulfuric acid (0.005 M), dilute 1.0 mL of 10 N sulfuric acid into 1.0 L of 18.2MΩ·cm ultrapure water (UPW). Volumetric preparation of this mobile phase will yield the most reproducible chromatography. See note below.

Note

It is advised to prepare sufficient mobile phase for the entire analysis to reduce the need to add additional mobile phase during an active sequence. Adding mobile phase during an active sequence may cause retention time shifting if the new mobile phase is not identical to the original mobile phase. This method uses roughly 18.0 mL of mobile phase per injection. Calculate how much mobile phase is needed before beginning analysis to prepare enough for the entire analysis.

2 Instrument equilibration

1. Purge instrument with 0.01 N sulfuric acid solution made in the previous step. Be certain the instrument is purged through the entire flow path including the detector, before the analytical column is plumbed in. The Aminex 87H column is sensitive to solvents, for example, a higher amount of methanol will damage the column. The purge step is needed to remove all solvents/mobile phases from the analytical system. (See Bio-Rad's '**Instruction Manual**' for Aminex resin-based columns for solvent compatibility and installation details).
2. Add the guard column and analytical column to the system and begin equilibrating the column at a low flow rate of 0.2 mL/min while the column reaches analysis temperature.
3. During the column equilibration, begin purging the reference cell of the refractive index detector (RID), this process will continue through the final equilibration of the column.
4. At intervals of at least 10 minutes, increase the flow by 0.2 mL/min until you reach the method flow of 0.6 mL/min.
5. Once the column is up to method flow and both the column compartment and the RID are at method temperature and stable, the RID reference cell can be closed. This typically takes around 30 minutes after method flow is reached. A longer purge of the reference cell is not detrimental.
6. After the reference cell is closed, wait for the RID signal to stabilize before starting analysis.

Preparation of standards

3 **Standards**

1. Prepare a 10 mg/mL ethylene glycol stock standard by weighing 50 mg of the ethylene glycol raw material into a 20 mL amber vial. Record the weight of the raw material to the nearest 0.1 mg. Add 5 mL of UPW to amber vial and vortex thoroughly until raw material is dissolved.
2. Repeat step 1 in order to create a 10mg/mL 1,4-butanediol stock standard.
3. Make separate calibration curves for ethylene glycol and 1,4-butanediol by following the calibration table below.

Calibration curve preparation for ethylene glycol and 1,4-butanediol

Calibration level	Concentration (µg/mL) (ppm)	Volume of 10 mg/mL stock standard (µL)	Volume of UPW as diluent (µL)	Total volume (µL)
1	25	100µL of level 4 (10x)	900	1000
2	50	100µL of level 5 (10x)	900	1000
3	100	100µL of level 7 (10x)	900	1000
4	250	25	975	1000
5	500	50	950	1000
6	750	75	925	1000
7	1000	100	900	1000
8	2500	250	750	1000
9	5000	500	500	1000
10	7500	750	250	1000
11	10000	1000	0	1000

example calibration curve preparation (click to enlarge)

Note

Reporting limits and linear ranges may vary and should be determined for each instrument individually. Stock standards can be made at varying concentrations that allow for quantification of samples within a desired range.

Preparation of samples

4 **Samples**

- Samples must be filtered through a 0.2 µm or smaller filter prior to injection on the HPLC ('Materials' section includes part numbers for filters to use depending on matrix composition)
- Samples expected to be over the linear range of the instrument should be diluted to ensure accurate analysis and avoid carryover.

HPLC-RID analysis

5 **Method Specifications**

Analysis is performed using an Agilent 1200 Series High Performance Liquid Chromatography (HPLC) system. An isocratic concentration of 0.01N sulfuric acid through an Aminex HPX-87H column (300 × 8.7 mm, 9 µm particle size) is used to achieve separation at a flow rate of 0.6 mL/min. Quantitation is determined using refractive index detection (RID). Column and RID are both held constant at 55 °C and each sample and standard is injected at a volume of 6.0 µL.

Binary pump configuration	
Flow rate	0.6 mL/min
Maximum pressure	100 bar
Mobile phase A	0.01N sulfuric acid (v/v)

Column compartment parameters	
Temperature	55 °C

Multisampler parameters	
Injection volume	6 µL
Draw speed	100 µL/min
Eject speed	100 µL/min
Wait time after draw	2 sec
Bottom sensing	enabled

Retention time of analytes is dependent on the configuration of the HPLC and will vary from instrument to instrument. Retention time markers for each analyte should be run individually to assess the total required run time of the analysis based on elution. Additional analytes not listed in this protocol may be compatible with these instrument parameters. This re-emphasizes the need to run single analyte retention time markers to prevent co-eluting peaks. The method run time is 27 minutes.

Note

Injection volume can be increased to 20 µL to obtain a lower reporting limit if necessary. This has the possibility to reduce the upper limit of quantitation due to signal saturation.

Analytical Quality Control

6 Multiple strategies are utilized when performing this analysis to ensure instrument stability and reproducibility.

6.1 **Calibration Curves**

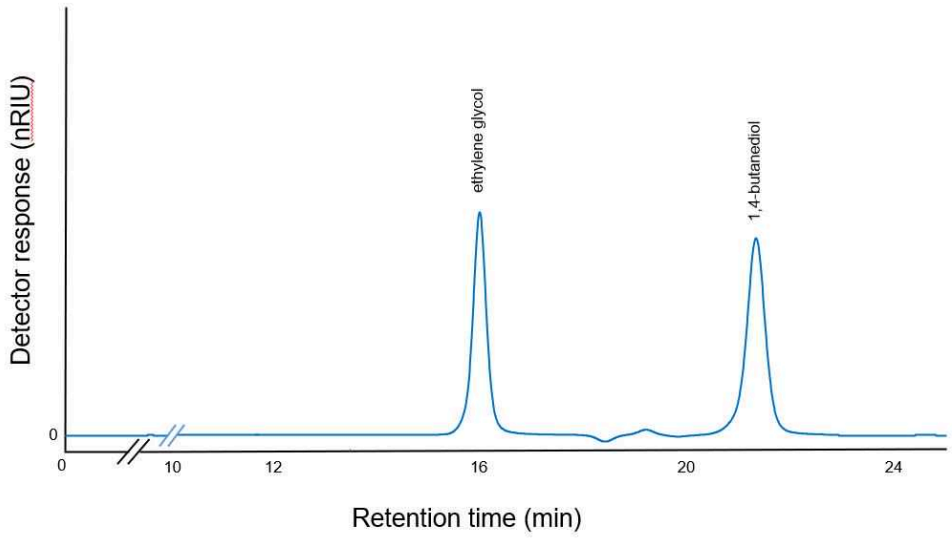
All compounds must have a correlation coefficient (r^2) of 0.995 or greater using a linear calibration fit and ignore the origin.

6.2 **Calibration Verification Standards (CVS)**

A calibration verification standard (CVS) is a level provided by the manufacturer that is re-analyzed every 20 or fewer samples to ensure instrument drift remains within the determined acceptance criteria. Acceptable CVS recoveries for this analysis are within 10% of the expected amount. Acceptance criteria may differ between instruments and should be determined experimentally.

Example Chromatography

7



Example chromatogram including elution order of ethylene glycol and 1,4-butanediol. (click to enlarge)