Application Note AN-C-196

Purity quantification of tris(hydroxymethyl)aminomethane (TRIS) with IC

Robust analysis with non-suppressed ion chromatography

Tris(hydroxymethyl)aminomethane (also known as TRIS, THAM, or tromethamine) is a common component of buffer solutions in the life sciences. It has a high buffering capacity between pH 7.2–9.0, a pK_a of 8.2 (20 °C), and complexes with metal ions, making TRIS ideal for biochemistry and molecular biology applications [1]. TRIS buffers are used for DNA purification, separation of proteins with SDS-PAGE (sodium dodecyl sulfate–polyacrylamide gel electrophoresis), or separation of nucleic acids with gel electrophoresis [2]. TRIS is also used to treat metabolic acidosis and can penetrate the cell membrane in its unionized form, therefore functioning as an intracellular buffer [3]. For these reasons, it is essential to control the purity of TRIS, especially for use in the pharmaceutical industry.

A robust isocratic ion chromatography (IC) method with a Metrosep C Supp 2 - 250/4.0 column and a methanesulfonic acid (MSA) eluent is ideally suited to determine TRIS and any cationic impurities. The microbore IC system (MB) is equipped with the IC Conductivity Detector MB which is both sensitive and stable against MSA eluents. This guarantees low void volumes, long-term stability of the analytical system, and precise results for TRIS quantification.

SAMPLE AND SAMPLE PREPARATION

Samples were prepared from Trizma® base (TRIS) powder with p.a. quality (CAS 77-86-1, purchased from Sigma Aldrich No. 93350). For method evaluation, two different concentrations of TRIS (10.37 mg/L and 103.7 mg/L) were dissolved in eluent (0.1% methanesulfonic acid).

EXPERIMENTAL

The microbore ion chromatograph 930 Compact IC Flex Oven/DEG/MB was equipped with the MSA-stable IC Conductivity Detector MB (Figure 1). An eluent consisting of 0.1% (v/v) MSA (15 mmol/L MSA) was used for this non-suppressed setup (Table 1). Samples were injected using the Metrohm intelligent Partial Loop Injection Technique (MiPT, Figure 2). This technique fills the 250 µL sample loop with a precisely measured and freely selectable volume (from 5 to 40 µL in this application study). During this process, a Dosino with a 2 mL Dosing Unit performs the precise dosing increments. MiPT enables calibration from a single standard, which was performed here in a range of 5 – 140 mg/L TRIS.

The variable volume selection can also be applied to sample injection. In such situations, a small injection volume is selected, e.g., for a highly concentrated sample therefore omitting the manual dilution step.

Typical inorganic cations (i.e., lithium, sodium, potassium, magnesium, and calcium) were injected on the Metrosep C Supp 2 column to check for potential co-elution issues.

Figure 1. The IC Conductivity Detector MB shown here has a reduced cell volume and is inert against methanesulfonic acid.

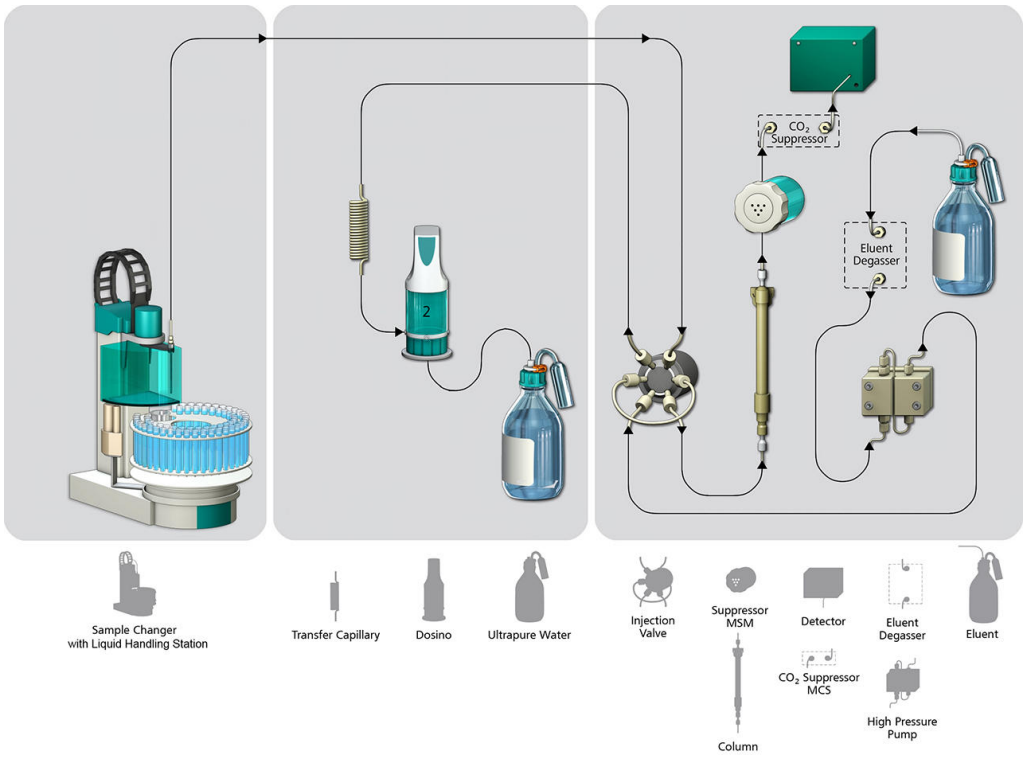

Figure 2. Illustration of the Metrohm intelligent Partial Loop Injection Technique (MiPT) flow path. With the help of the Dosino, the sample is transferred from the autosampler into a buffer loop to avoid contamination and carryover. The Dosino then precisely fills the sample loop with the desired injection volume in the µL range.

Table 1. IC method parameters for the microbore IC analysis of cationic impurities in TRIS.

Column	Metrosep C Supp 2 - 250/4.0
Eluent/diluent	c(MSA) = 0.1 % (v/v)
Flow rate	1.0 mL/min
Temperature	30 °C
Injection volume	5–40 µL (MiPT)
Detection	Direct conductivity

RESULTS

TRIS determination is carried out in less than 8 min using isocratic elution on the MB IC system. The method was proven to be interference-free with regard to major cations as described above.

Sodium had a retention time of 4.1 minutes. Changing the method parameters, e.g., decreasing the column temperature to 20 °C, will increase the resolution between sodium and TRIS. With the used method parameters (Table 1), precise determination is possible by using the peak height for evaluation. The recovery rates for 100 mg/L TRIS were 99–103% with a relative standard deviation of <3%, revealing the accuracy of this method.

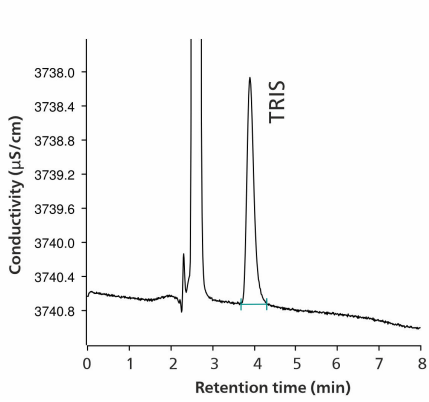

Figure 3. Chromatogram of 100 mg/L TRIS with 4 µL injection volume (MiPT).

CONCLUSION

Raw materials used in the pharmaceutical industry like solutions and buffers must fulfill the highest quality standards with respect to their exact concentration and purity.

The setup in this application study comprises a microbore IC system, an MSA-stable conductivity detector, and MiPT for automatic calibration with a single standard and flexible choice of sample injection volumes. The method is suitable for the quantification of TRIS in the range of 5 – 200 mg/L. It guarantees robust determination of the common buffer component TRIS in an easy and precise way.

REFERENCES

1. Deutscher, M. P. *Guide to Protein Purification*; Gulf Professional Publishing, 1990.
2. Westermeier, R. *Electrophoresis in Practice: A Guide to Methods and Applications of DNA and Protein Separations*; John Wiley & Sons, 2016.
3. Sirieix, D.; Delayance, S.; Paris, M.; et al. Tris-Hydroxymethyl Aminomethane and Sodium Bicarbonate to Buffer Metabolic Acidosis in an Isolated Heart Model. *Am J Respir Crit Care Med* **1997**, *155* (3), 957–963. https://doi.org/10.1164/ajrccm.155.3.9117032.

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CONFIGURATION

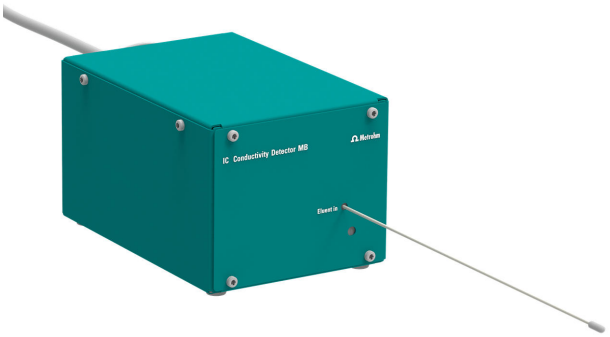
Compact IC Flex Oven/SeS/PP/Deg/MB

The 930 Compact IC Flex Oven/SeS/PP/Deg is the intelligent Compact IC instrument with **column oven, sequential suppression**, a **peristaltic pump** for suppressor regeneration and a built-in **degasser**. The instrument can be used with any separation and detection methods.

Typical areas of application:

- Anion or cation determinations with sequential suppression and conductivity detection
- Optimized for microbore (2 mm) applications, ideally suitable for coupling techniques (IC-MS or IC-ICP/MS)

Supported with MagIC Net 4.1 and higher



IC Conductivity Detector MB

Compact and intelligent high performance conductivity detector for intelligent IC instruments. Optimized for microbore columns. Outstanding temperature stability, the complete signal processing within the protected detector block and the latest generation of DSP – Digital Signal Processing – guarantee the highest precision of the measurement. No change of measuring ranges (not even automatic ones) is required, due to the dynamic working range.

Typical areas of application:

- Anion or cation determinations with chemical suppression, sequential suppression or without suppression and conductivity detection
- Optimized for microbore (2 mm) applications, ideally suitable for coupling techniques (IC-MS or IC-ICP/MS)

Specification at a glance:

- 0–15000 µS/cm without range switching
- Cell volume: 0.3 µL
- Ring-shaped electrodes made of stainless steel X2CrNiMo17-12-2 (316 L), compatible with MSA
- Maximum operating pressure: 10.0 MPa (100 bar)
- Cell temperature: 20–50 °C in increments of 5 °C
- Temperature stability: < 0.001 °C
- Baseline noise: < 0.2 nS/cm typical for sequential suppression
- Capillaries: ID 0.18 mm

Supported with MagIC Net 4.1 and higher

Metrosep C Supp 2 - 250/4.0

The longest separation column in the Metrosep C Supp 2 product range is the Metrosep C Supp 2 - 250/4.0. The Metrosep C Supp 2 separation material is based on a polystyrene-divinylbenzene copolymer with carboxyl groups. Thanks to the optimized sodium/ammonium separation of this separation material, this column is perfectly suitable for determination of the smallest concentrations of ammonium in addition to a large amount of sodium. The column is used with sequential suppression. So it is particularly suitable for determining concentrations in the middle µg/L range and lower.