Application Note AN-C-196

使用子色法(IC)三基甲基基甲(TRIS)行度定量分析

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.

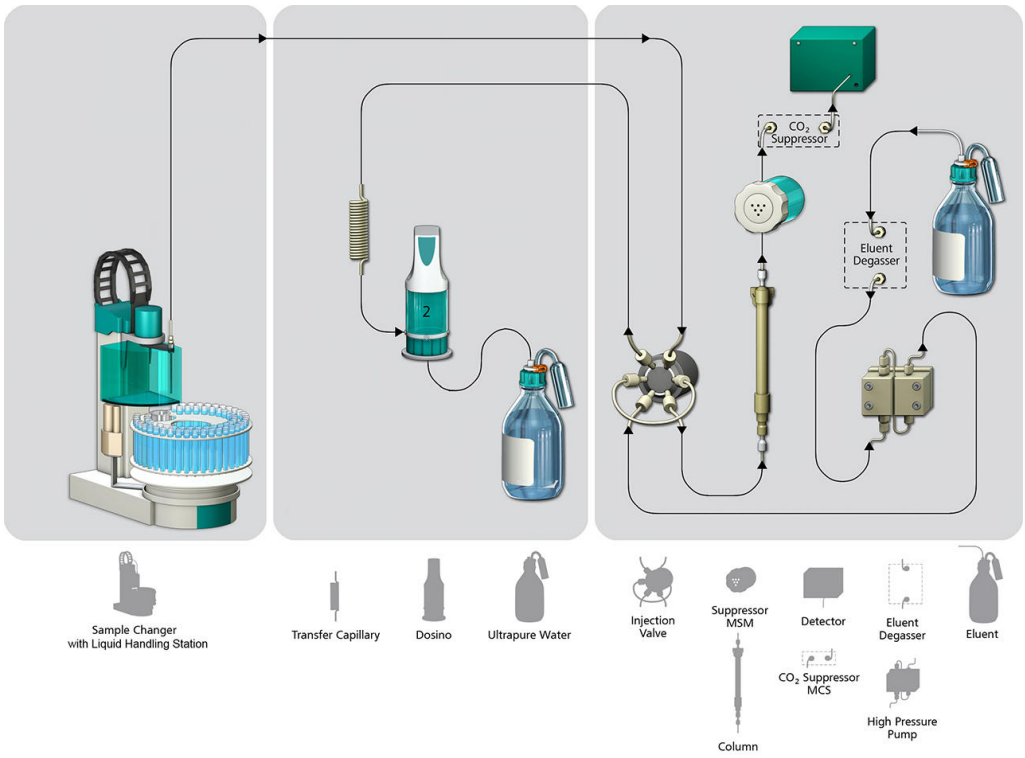


Figure 2. Illustration of the Metrohm intelligent Partial Loop Injection Technique (MiPT) flow path. With the help of the Dosino, the sample is transferred from the autosampler into a buffer loop to avoid contamination and carryover. The Dosino then precisely fills the sample loop with the desired injection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

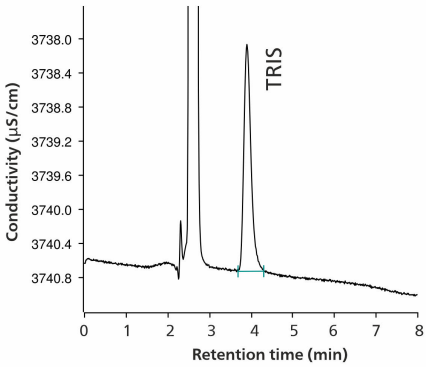


Figure 3. Chromatogram of 100 mg/L TRIS with 4 µL injection volume (MiPT).

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.

CONTACT

瑞士万通中国
北京市海淀区上地路1号院
1号楼7702
100085 北京
marketing@metrohm.com.cn

CONFIGURATION

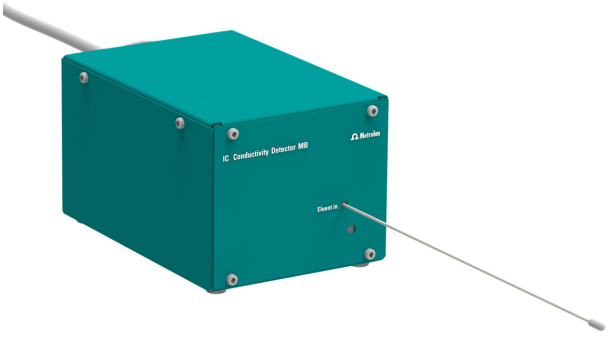
930 Compact IC Flex Oven/SeS/PP/Deg/MB

930 Compact IC Flex Oven/SeS/PP/Deg 是智能型 Compact 子色器,有**柱加炉、序列抑制**和用于抑制器再生的**蠕**,以及内置的**脱气装置**。器可使用任意分和方法。

典型的用范:

- 子或子定,序列抑制法及
- 微孔 (2mm) 用化,尤其合合技(IC-MS 或 IC-ICP/MS)

支持 MagIC Net 4.1 和以上版本



IC Conductivity Detector MB

用于智能型子色的智能型高性能器。微孔柱化。卓越的温度定性,在受保的器端子板内完成整个信号理程以及最新一代的 DSP(数字式信号理)均能保量的最高精性。功于工作范,无需行(也包括非自)范更。

典型的用范:

- 子或子定,化学抑制、序列抑制法或无抑制及
- 微孔 (2mm) 用化,尤其合合技(IC-MS 或 IC-ICP/MS)

格明概:

- 0 ... 15000 µS/cm 无区段切
- 量池容量:0.3 µL
- 由不 X2CrNiMo17-12-2 (316 L) 制成的形,与 MSA 兼容
- 最大工作力:10.0 MPa (100 bar)
- 池温:20 ... 50 °C,步幅 5 °C
- 温度定性:< 0.001 °C
- 基噪音:<° 0.2° nS/cm,是使用序列抑制法的典型
- 毛管:ID 0.18 mm

支持 MagIC Net 4.1 和以上版本

Metrosep C Supp 2 - 250/4.0

Metrosep C-Supp-2 系列的分柱是 Metrosep C Supp 2 - 250/4.0。Metrosep C-Supp-2 分材料基于一基的聚乙-二乙共聚物。由于分材料佳的/分,故此柱非常用于除大量以外的小度定。柱可以搭配序列抑制法使用。故此,其特用于定平均 µg/L 范及以下的度。