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.

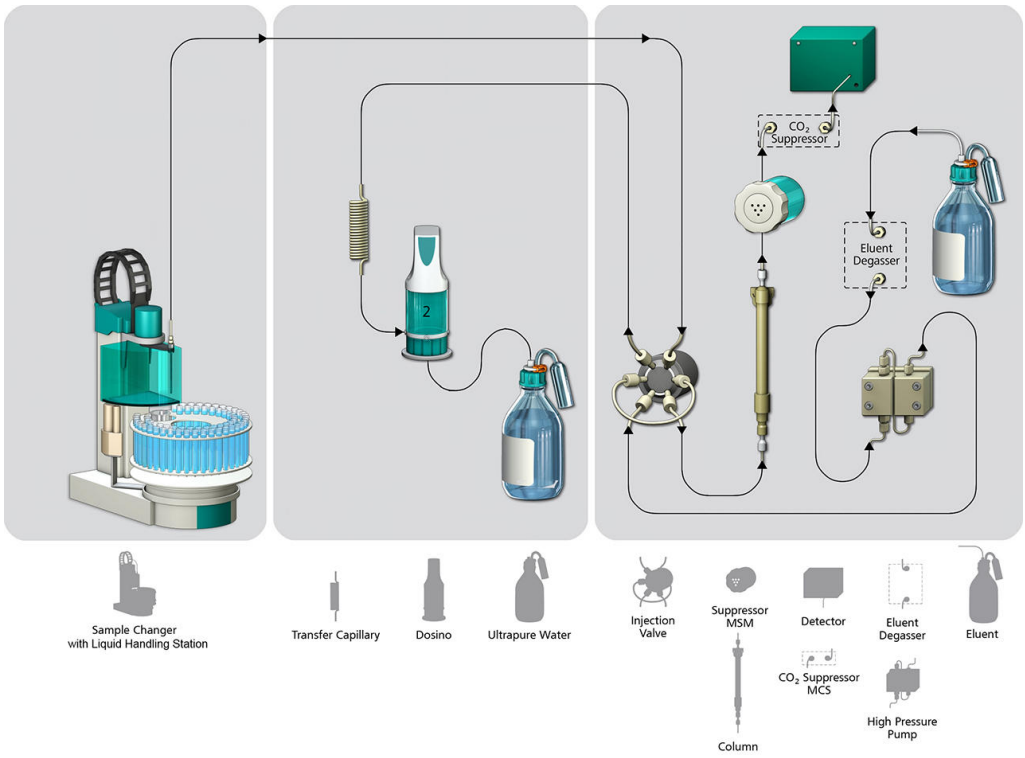

Figure 2. Illustration of the Metrohm intelligent Partial Loop Injection Technique (MiPT) flow path. With the help of the Dosino, the sample is transferred from the autosampler into a buffer loop to avoid contamination and carryover. The Dosino then precisely fills the sample loop with the desired injection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

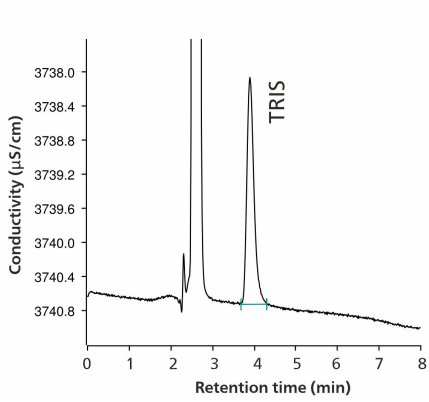

Figure 3. Chromatogram of 100 mg/L TRIS with 4 µL injection volume (MiPT).

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

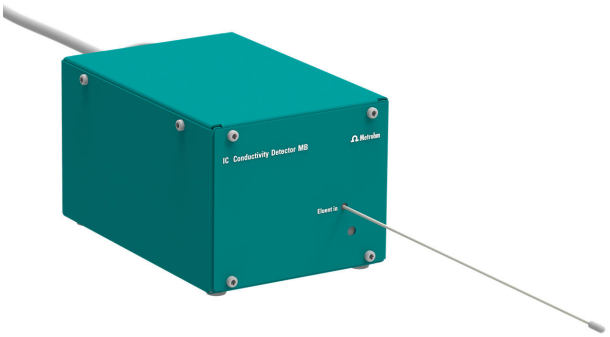
930 Compact IC Flex Oven/SeS/PP/Deg/MB

930 Compact IC Flex Oven/SeS/PP/Deg は**カラムオーフン、連続サフレッション**、サフレッサー再生のための**ヘリスタリックホンフ**、内蔵式**脱気装置**を備えたインテリシェントなコンハクトIC 装置てす。この装置は任意の分離メソットおよび検出メソットによって使用することかてきます。

典型的な使用領域:

- 電気伝導度検出器による、連続サフレッションされる陰イオンまたは陽イオンの測定
- マイクロホア (2mm) アフリケーション向けに最適化、カッフリンク技術 (IC-MS または IC-ICP/MS)に最適

MagIC Net 4.1 以上に対応



IC Conductivity Detector MB

インテリシェントIC装置のためのコンハクトかつインテリシェントな高出力電気伝導度検出器。マイクロホアカラム向けに最適化。優れた温度安定性、保護された検出器フロック内の総合的な信号処理、最新版の DSP (Digital Signal Processing) か高精度の測定を保証します。稼動範囲かタイナミックなのて測定範囲の変更は(自動のものも含めて)不必要てす。

典型的な使用領域:

- 電気伝導度検出器による、化学的サフレッションまたは連続的な化学的サフレッションのある、もしくはサフレッション無しの陰イオンおよび陽イオンの測定
- マイクロホア (2mm) アフリケーション向けに最適化、カッフリンク技術 (IC-MS または IC-ICP/MS)に最適

仕様概要:

- 0~15000 µS/cm、エリアの切り替えなし
- セル容量: 0.3 µL
- リンク状のステンレス製電極 X2CrNiMo17-12-2 (316 L)、MSA と互換
- 最大運転圧力: 10.0 MPa (100 ハー)
- セル温度: 20~50° C、5° C刻み
- 温度安定性: < 0.001° C
- ヘースラインノイス: < 0.2 nS/cm、連続サフレッションの平均値
- キャヒラリー: ID 0.18 mm

MagIC Net 4.1以上に対応

Metrosep C Supp 2 - 250/4.0

Metrosep C-Supp-2のシリースて最も長い分離カラムは、Metrosep C Supp 2 - 250/4.0てす。Metrosep C-Supp-2分離材料は、カルホキシル基を含むホリスチレン・シヒニルヘンセン共重合体をヘースとしています。最適化された分離材料のナトリウム/アンモニウム分離のおかけて、このカラムは非常に多量のナトリウムに加えて、最も低いアンモニウム濃度の測定にも非常に良く適しています。カラムは、連続サフレッションを伴って使用されます。従って、特に中程度のµg/L範囲およひそれ以下の濃度の測定に適しています。