Trace-level aliphatic amines in cationic pharmaceutical ingredients

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Ion Chromatography

Summary

The analytical challenge treated by the present work consists in detecting sub-ppb concentrations of low-molecular-weight amines in the presence of strongly retained cationic drugs by using ion chromatography (IC) with upstream inline coupled-column matrix elimination (CCME). In contrast to direct-injection IC, where the late elution of strongly retained drugs requires eluents with added acetonitrile, the CCME technique uses two preconcentration columns in series. In an «inverse matrix elimination» step, cationic drug and target amines are trapped on a high-capacity and a very-high-capacity preconcentration column, respectively. During amine determination, a rinsing solution flushes the drug to waste. This significantly shortens the analysis time and improves sensitivity as well as selectivity. Besides the determination of monomethylamine in Nebivolol hydrochloride discussed here, the CCME technique is a promising tool for detecting further low-molecular-weight amines in a wide range of drugs.

Introduction

Low-molecular-weight amines find widespread use in raw materials or intermediate products, in the manufacturing of numerous chemicals, pharmaceuticals, polymers, pesticides, rubber, dyes, adhesives, solvents and corrosion inhibitors. Their monitoring is crucial as most of them are toxic and irritate the skin, mucous membranes and respiratory tract. Moreover, secondary amines can react with nitrite forming carcinogenic nitrosamines. After-exposure determination of trace-levels of aliphatic amines in body fluids is therefore of utmost interest for the evaluation of metabolic patterns and for biological monitoring.

However, the determination of low-molecular-weight amines in interfering matrices is a challenging task because the protonated amines are often poorly retained on the column, which results in very short retention times, poor separation and strongly asymmetric peaks. Aliphatic amine monitoring in pharmaceuticals requires ultra-trace amine determination that is additionally hampered by the presence of interfering cationic pharmaceutical ingredients.

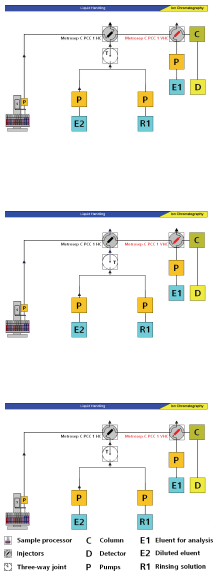
This problem can be overcome by applying the coupled-column approach: After trapping the aliphatic amines and the interfering pharmaceutical on a high-capacity preconcentration column, only the amines are selectively eluted and transferred to a very-high-capacity preconcentration column. Prior to flushing the interfering drug to waste, the amine fraction is transferred to the separation column proper.

This poster deals with the determination of monomethylamine in cationic drugs using straightforward CCME. After optimization of system parameters, trace level of monomethylamine (MMA) are determined in the cationic beta blocker Nebivolol hydrochloride.

Instrumentation

- 850 Professional IC Cation – Prep 2
- 858 Professional Sample Processor
- Metrosep C PCC 1 HC
- Metrosep C PCC 1 VHC

Coupled-column matrix elimination (CCME)



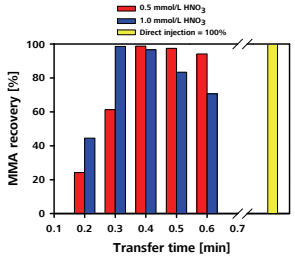
The sample is transferred to the C PCC 1 HC high-capacity preconcentration column where the amines and the cationic drug are trapped. Sample transfer occurs by a peristaltic pump using ultra-pure water that was previously freed of trace cations by passing through a high-capacity trap column.

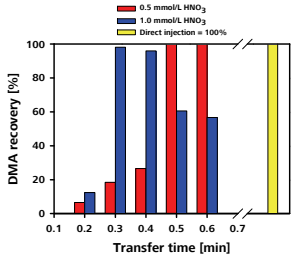
While the cationic drug is retained on the C PCC 1 HC, amines are selectively eluted with eluent E2 and subsequently trapped on the C PCC 1 VHC very-high-capacity preconcentration column. Eluent composition E2 and corresponding transfer time were optimized in preliminary experiments.

Trapped amines are eluted with eluent E1 and transferred to the separation column. Simultaneously, the rinsing solution R1, having a high organic modifier content, flushes the drug from the cation-retaining C PCC 1 HC to waste. The C PCC 1 HC is now ready for the next sample.

Optimization of eluent composition E2 and transfer time

By means of the early-eluting monomethylamine (MMA) and dimethylamine (DMA), eluent composition E2 and the corresponding transfer time were optimized in terms of amine recovery. The latter was calculated by comparing the peak area obtained after matrix elimination with the peak area after direct injection onto the preconcentration column C PCC 1 VHC.



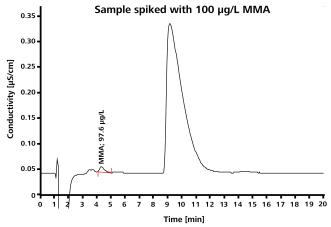


Apart from MMA and DMA, the CCME technique was optimized for ethylamine, diethylamine, n-propylamine, n-butylamine and benzylamine.

MMA in Nebivolol hydrochloride

By means of MMA determination in Nebivolol hydrochloride – an exemplary cationic drug and cardioselective beta blocker with pronounced antihypertensive effect – the performance of the common direct-injection procedure and of the previously described CCME are illustrated.

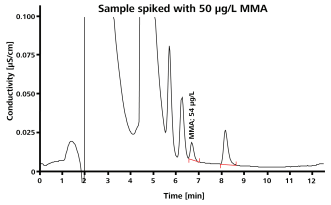
a) Direct injection

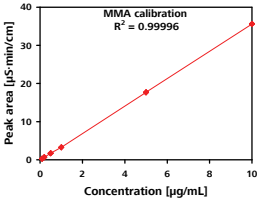


Column:	Metrosep C 4 – 150
Column temp.:	25 °C
Eluent E1:	2 mmol/L nitric acid, 40% acetonitrile as organic modifier
Eluent E2:	-
Transfer time:	-
Flow:	1 mL/min
Running time:	25 minutes

Acetonitrile additions to the eluent reduce the strong lipophilic interactions of the cationic pharmaceutical with the column`s carrier material and thus shorten the drug`s retention time. However, such organic solvent additions impair detection sensitivity and selectivity. While direct injection is still feasible for MMA determination in Nebivolol hydrochloride, monitoring of higher amine homologues in strongly retained cationic drugs becomes increasingly difficult.

b) CCME





Column:	Metrosep C 3 – 250
Column temp.:	25 °C
Eluent E1:	2 mmol/L nitric acid, no organic modifier
Eluent E2:	1 mmol/L nitric acid
Transfer time:	0.35 min
Flow:	1 mL/min
Running time:	10 minutes

	0.05 µg/mL spike		0.1 µg/mL spike		1.0 µg/mL spike	
	Determined [µg/mL]	Recovery [%]	Determined [µg/mL]	Recovery [%]	Determined [µg/mL]	Recovery [%]
1	0.049	98.0	0.109	109.0	1.017	101.7
2	0.054	108.0	0.107	107.0	1.015	101.5
3	0.052	104.0	0.103	103.0	1.014	101.4
Mean		**103.33**		**106.33**		**101.53**
SD		5.033		3.055		0.153
RSD		4.87%		2.87%		0.15%

After optimization of the chromatographic conditions, the presented CCME method allows the accurate simultaneous determination of various low-molecular-weight amines in strongly retained cationic drugs.

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