Determining Trace-Level Aliphatic Amines in Cationic Pharmaceuticals

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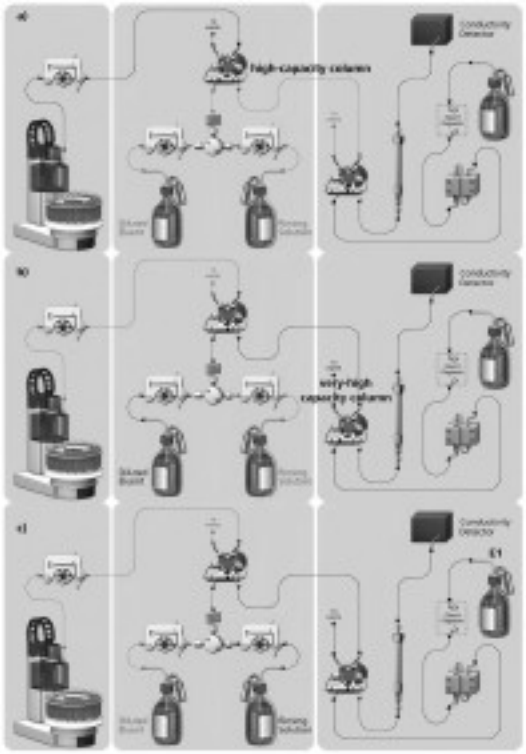
Coupled-column matrix elimination (CCME) offers a promising tool for the determination of low-molecular-weight amines in a wide range of drugs.

The analytical challenge discussed in this article consists of determining 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 is a significant drawback and requires eluents with added acetonitrile, the low-pressure CCME technique – by using two preconcentration columns in series – gets by without solvent-containing eluents. 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 the separation step, a rinsing solution flushes the drug retained on the high-capacity preconcentration column to waste. The application of CCME significantly shortens analysis time, protects the analytical column and improves sensitivity as well as selectivity.

In the example presented here, the CCME technique is used to determine trace levels of monomethylamine (MMA) in the cationic beta-blocker nebivolol.

Figure 1: Schematic illustration of the low-pressure coupled-column matrix elimination. The high-capacity (preconcentration) column is a Metrosep C PCC 1 HC/4.0 and the very-high-capacity (preconcentration) column is a Metrosep C PCC 1 VHC/4.0



THE CHALLENGE OF LOW-MW AMINES

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.

Their determination, however, 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. Separations become even more demanding in the presence of interfering cationic drug ingredients.

These shortcomings can be overcome by applying CCME. After optimisation of the CCME parameters, trace levels of MMA are determined in the cationic beta blocker nebivolol hydrochloride. Alternatively, direct injection results are presented.

EXPERIMENTAL ANALYSIS

Upstream matrix elimination uses a high-capacity and a very-high-capacity preconcentration column in series. Three peristaltic pumps (see Figure 1) take over the complete sample transfer steps within the CCME. All determinations were performed on the Metrohm 850 Professional IC – Cation – Prep 2. Chromatographic conditions are indicated in the respective chromatograms.

All reagents used in this work were of the highest purity grade. Eluents were prepared using deionised water with a specific resistance higher than 18 MΩcm. While supra-pure nitric and hydrochloric acid for eluent preparation were obtained from Merck/Germany, analytical grade acetonitrile was purchased from Merck/India. Amines and their respective hydrochloride salts were purchased from Sigma-Aldrich (Switzerland, Fluka brand). Nebivolol hydrochloride samples originated from Heterolab (Hyderabad, India).

Coupled-Column Matrix Elimination

First, the sample is transferred to the high-capacity (preconcentration) column, which traps the amines and the cationic drug (see Figure 1a). Sample transfer occurs by a peristaltic pump using ultrapure water that was previously freed of trace cations by passing through a trap column.

Secondly, while the cationic drug is retained on the high-capacity (preconcentration) column, amines are selectively eluted with the diluted eluent and subsequently trapped on the very-high-capacity (preconcentration) column (see Figure 1b). The optimisation of the composition of the diluted eluent and corresponding transfer time is described below.

Thirdly, the trapped amines are eluted with eluent E1 and transferred to the separation column (see Figure 1c). Simultaneously, a rinsing solution, having a high organic

Figure 2: Optimisation of the composition of the diluted eluent and transfer time for: A) MMA and B) DMA

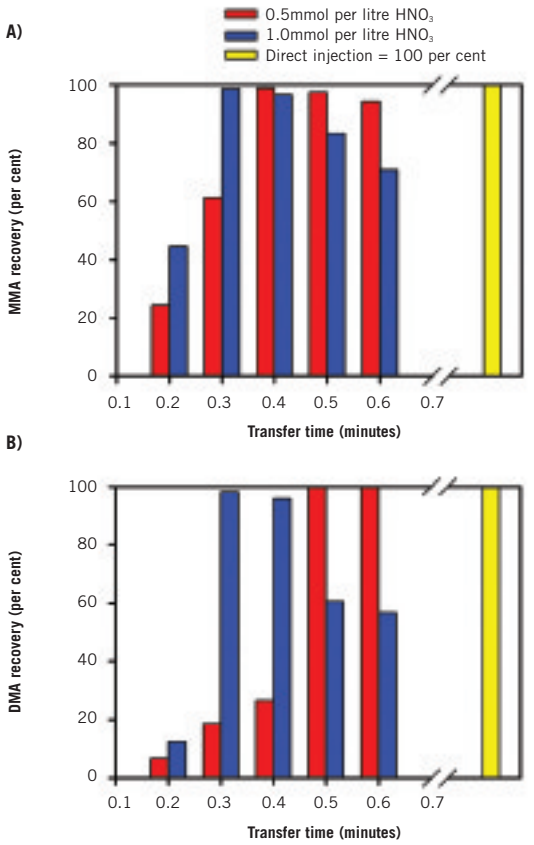
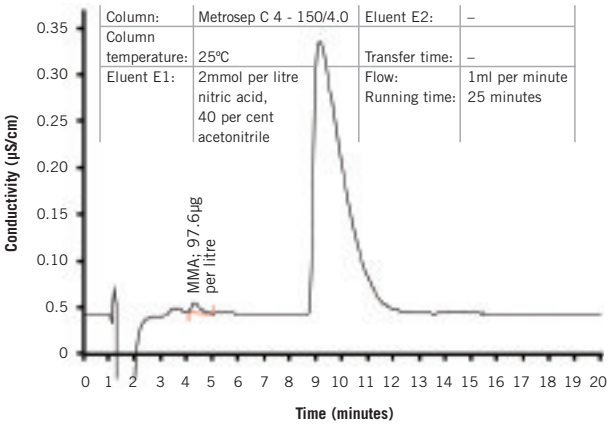


Figure 3: Direct-injection chromatogram of nebivolol hydrochloride spiked with 100µg/L MMA



modifier content, flushes the drug from the cation-retaining high-capacity (preconcentration) column to waste. This column is now ready for the next sample.

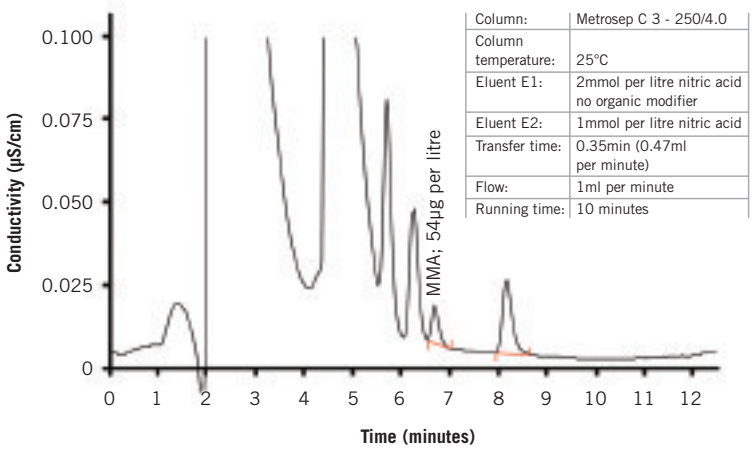
Optimisation of the Composition of the Diluted Eluent and of the Transfer Time

By means of the early-eluting MMA and dimethylamine (DMA), the composition of the diluted eluent and the

Table 1: MMA recoveries in nebivolol hydrochloride samples

	0.05mg/L MMA spike		0.1mg/L MMA spike		1.0mg/L MMA spike	
	Determined [mg/L]	Recovery [per cent]	Determined [mg/L]	Recovery [per cent]	Determined [mg/L]	Recovery [per cent]
1	0.049	98.0	0.109	109	1.017	101.7
2	0.054	108.0	0.107	107	1.015	101.5
3	0.052	104.0	0.103	103	1.014	101.4
Mean		**103.33**		**106.33**		**101.53**
RSD		**4.87 per cent**		**2.87 per cent**		**0.15 per cent**

Figure 4: Chromatogram of nebivolol hydrochloride spiked with 50µg/L MMA after upstream CCME



corresponding transfer time (flow rate = 0.47mL/min) were optimised 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 very high (preconcentration) column.

According to Figure 2 (page 17), the best results for MMA and DMA determination were obtained for 1.0mmol/L nitric acid as diluted eluent and a transfer time of 0.35 minutes.

MMA in Nebivolol Hydrochloride

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

- **Direct Injection** – 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. Such organic solvent additions, however, impair detection sensitivity and selectivity. While direct injection is still feasible for MMA determination in nebivolol hydrochloride (see Figure 3, page 17), monitoring of higher amine homologues in strongly retained cationic drugs becomes increasingly difficult.

- **CCME** – allows for a rapid determination of trace-level MMA with recoveries ranging between 101 and 106 per cent (see Table 1). Relative standard deviations (RSDs) are better than five per cent.

CONCLUSION

The CCME method excels by its capability to simultaneously determine various low-molecular-weight amines in strongly retained cationic drugs. Besides the determination of monomethylamine in nebivolol hydrochloride, as discussed above, the CCME technique is a promising tool for determining further low-molecular-weight amines in a wide range of drugs.

References

1. **Harihara Subramanian N, Development of novel methods for the determination of trace ionic contaminants in complex matrices using ion chromatography, PhD thesis, University of Chennai, India, 2009**
2. **Metrohm Application Notes AN-C-007, AN-C-052, AN-C-057, AN-C-070; AN-C-078, AN-C-092, AN-C-093 and AN-C-124, AN-C-126, http://products.metrohm.com**

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