Monomethylamine (MMA), like other aliphatic amines, has been applied at various stages of the synthesis of many active substances such as, for example, tadalafil, didanosine, alfuzosin hydrochloride, sertraline or nebivolol hydrochloride which has been studied in this paper. Currently, nebivolol, a third-generation selective β-blocker of major therapeutic interest, is a widely-used drug for treatment of many cardiovascular diseases. This drug offers greatest hope for the effective treatment and there are no side effects reported which are characteristic of other β-adreno lytic drugs.

Aliphatic amines such as MMA, monoethylamine (MEA) and other short-chain amines are important intermediates in chemical and pharmaceutical industries. MMA is widely used in different stages of synthesis of various drug substances.

The reason for interest in the MMA determination is a possibility of contamination of the latter by toxic MMA. Therefore, it is essential to determine the level of the MMA in the active substances before they are released for manufacturing of medical products. For this purpose, the use of simple analytical methods with satisfactory sensitivity allows determination of methylamine at the ppm level.

The literature describes determination of MMA in so-called environmental samples which were taken from: air (1-5), water and sewage (6-10) and food (11-14), because the widespread use of aliphatic amines in industry may have a significant environmental negative impact. Less studies relating to the determination of methylamine in biological material (15-17), pharmaceutical formulations (18), active substances (19), or in diagnostics (20) were found.

A wide range of techniques allowing the determination of methylamine is described in the literature – from most common used chromatographic methods GC or HPLC to novel determination techniques based on the using of biosensors (4, 15).

Gas chromatography is suitable for the separation of volatile compounds such as short-chain...
aliphatic amines. These techniques with different types of detectors, flame ionization detector (2) and MS detector (6-8, 11) have been successfully employed for many years. A number of HPLC methods have also been developed for the determination of aliphatic amines, including methylamine, after their derivatization using various reagents. Such detection methods as fluorescence (9, 12, 16, 17), UV (1) and mass detection (10, 14) have been used. One of the HPLC methods, ion chromatography HPIC was applied for the determination of MMA with conductivity detection: without conductivity suppression (3, 5, 19) or with the electrochemically suppressed conductivity detection reported in only one elaboration of the Thermo Scientific company, posted on the web in 2013 (18).

The aim of this study was to develop a simple and environmentally friendly method for the determination of MMA as an impurity originated from the synthesis of nebivolol hydrochloride. The analysis was performed using the high-performance ion chromatography (HPIC) method with the suppressed conductivity detection.

Compared to the method described by Thermo Scientific (19) we report a less complicated chromatographic system with isocratic elution and electrochemically generated eluent replaced by a simple eluent such as 10 mM methanosulfonic acid (MSA) and chemical (membrane) suppression. Apart from the methylamine, the presented method has also been used for twelve other short-chain aliphatic amines and alkanolamines.

EXPERIMENTAL

Chemicals, reagents

Monomethylamine (MMA) water solution 40.6% (analytical grade) was used as a standard substance.

The following chemicals (analytical grade) were used: aluminium nitrate nonahydrate, ammonium acetate, barium nitrate, calcium acetate hydrate, cesium chloride, diethylamine, disopropylamine, N,N-diisopropylethylamine, dimethylamine, ethylenediamine, isopropylamine, magnesium chloride hexahydrate, monoethanolamine, monoethylene glycol, polyethylene glycol (MEA), methanesulfonic acid (MSA), potassium chloride, triethanolamine, triethylamine, trimethylamine, sodium chloride; tetrabutylammonium hydroxide (TBA) solution ~40% in water (for ion chromatography); syringe membrane filters with: glass fibre-pore size 1.0 μm (Chromafil GF-100/25), polyvinylidene difluoride - pore size 0.45 μm (Chromafil Xtra PVDV 45/25). Mili-Q water (= 18 MΩ) was used for mobile phase preparation.

Equipment

The ion chromatography system ICS-90 ( Dionex Co., USA) controlled by a computer, with conductivity detector DS5, Cation MicroMembrane Suppressor CMMS III (4 mm) and sample injection valve equipped with 100 μL loop. The software used for data processing and chromatography was Chromeleon 6.50. The following programs were used for statistical evaluation: EXCEL 98 and Chromeleon 6.50. Separation was achieved using IonPac CS14 (250 × 4 mm) column and IonPac CS14 (50 × 4 mm) precolumn.

RESULTS AND DISCUSSION

Method development

As a first step, a search was carried out for an optimal HPIC system, which would enable a good separation of MMA from other aliphatic amines.

The employment of the most common HPIC detection technique using conductivity detector with suppression allowed to obtain high sensitivity and detection of other aliphatic amines beside methylamine. Due to their properties (pKa value is about 10), most aliphatic amines sufficiently dissociate in aqueous solutions, which enables them to reach the detector in the cationic form.

The ICS-90 ion chromatograph with membrane suppressed conductivity detection was applied. The suppression of eluent conductance enhances the sensitivity, but it causes some limitations when it comes to the choice of the mobile phase. In practice, in the case of detection of cations, the choice of eluents to be used in this technique is limited to diluted solutions of strong acids, such as sulfuric, hydrochloric or nitric acid.

Macroporous-type weak cation-exchange resin containing carboxylic acid groups located directly on the surface or in the pores (100 Å) of ethylvinylnitrobenzene-divinylbenzene copolymer (55% cross-linked) was used as a stationary phase. Although using such stationary phases makes the analysis of concentrated samples impossible due to low column capacity, it considerably reduces the analysis time (almost twice) with respect to microporous phases.

A further advantage is the high chemical stability of such column filling materials.

The carboxylic acid groups, relatively weak ion-exchangers, make possible separation of the majority of monovalent and divalent cations under
isocratic conditions using mobile phases with weak elution strength, such as methanesulfonic acid. The relatively high hydrophobicity of the matrix (ethylvinylbenzene-divinylbenzene copolymer), enables the separation of most alkyl- and alkanolamines with satisfactory efficiency due to minimizing secondary adsorption.

Validation of the method

Monomethylamine (MMA) determination in nebivolol hydrochloride active substance was carried out with the HPIC method under the following conditions:
- Detection: conductivity with a cation micromembrane suppressor
- Column: IonPac CS14 (250 × 4.0 mm)
- Precolumn: IonPac CS14 (50 × 4.0 mm)
- Flow rate: 1.0 mL/min
- Column temperature: ambient
- Mobile phase: 10 mM methanesulfonic acid (MSA)
- Injected volume: 100 µL
- Suppressor: CMMS III (4 mm)
- Regeneration: 100 mM tetrabutylammonium hydroxide (TBA)
- Background conductivity: 7-8 µS

Selectivity

Chromatograms of selected alkyl- and alkanolamines and common metal ions were recorded. Nebivolol hydrochloride was not eluted under the designated chromatographic conditions. The retention times of analyzed cations are shown in Table 1.

Linearity

Linearity was successfully tested within a concentration range 0.03-2.4 µg/mL of monomethylamine. The results are shown in Table 2.

Detection and quantification limits

Limit of detection (LOD) and a limit of quantification (LOQ) were established based on the signal to noise ratio 3 × S/N and 10 × S/N, respectively. The results are presented in Table 2.

Precision

In order to estimate the system precision, the standard solutions of monomethylamine with the concentration of 0.03 µg/mL (LOQ) were injected six times into the column.

RSD of the peak area was found to be 3.5%.

<table>
<thead>
<tr>
<th>Cation</th>
<th>Retention time (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium</td>
<td>3.7</td>
</tr>
<tr>
<td>Ammonium</td>
<td>4.2</td>
</tr>
<tr>
<td>Monoethanolamine</td>
<td>4.6</td>
</tr>
<tr>
<td><strong>Monomethylamine (MMA)</strong></td>
<td><strong>4.6</strong></td>
</tr>
<tr>
<td>Monoethanolamine (MEA)</td>
<td>4.8</td>
</tr>
<tr>
<td>Potassium</td>
<td>5.4</td>
</tr>
<tr>
<td>Dimethylamine</td>
<td>5.5</td>
</tr>
<tr>
<td>Triethanolamine</td>
<td>5.7</td>
</tr>
<tr>
<td>Trimethylamine</td>
<td>6.0</td>
</tr>
<tr>
<td>Isopropylamine</td>
<td>6.3</td>
</tr>
<tr>
<td>Diethylamine</td>
<td>6.6</td>
</tr>
<tr>
<td>Cesium</td>
<td>7.2</td>
</tr>
<tr>
<td>Magnesium</td>
<td>8.3</td>
</tr>
<tr>
<td>Calcium</td>
<td>9.2</td>
</tr>
<tr>
<td>Aluminium</td>
<td>9.3</td>
</tr>
<tr>
<td>Diisopropylamine</td>
<td>13.2</td>
</tr>
<tr>
<td>Barium</td>
<td>14.5</td>
</tr>
<tr>
<td>Triethylamine</td>
<td>16.1</td>
</tr>
<tr>
<td>N,N-diisopropylethylamine</td>
<td>43.0</td>
</tr>
<tr>
<td>Ethylenediamine</td>
<td>46.2</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Statistical parameters</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Line equation (n = 6)</td>
<td>$y = 5.552 \times 10^4 - 5.668 \times 10^3$</td>
</tr>
<tr>
<td>Concentration range (µg/mL)</td>
<td>0.03-2.4</td>
</tr>
<tr>
<td>$Y_{	ext{intercept}}$ (%)</td>
<td>0.42</td>
</tr>
<tr>
<td>Correlation coefficient (r)</td>
<td>0.9999</td>
</tr>
<tr>
<td>Limit of detection LOD (µg/mL)</td>
<td>0.01</td>
</tr>
<tr>
<td>Limit of quantification LOQ (µg/mL)</td>
<td>0.03</td>
</tr>
<tr>
<td>Precision for limit of quantification LOQ (n = 6); RSD (%)</td>
<td>3.5</td>
</tr>
</tbody>
</table>
RSD <10% was used as an acceptance criterion. In addition, precision for solutions at concentrations higher than LOQ was also confirmed. RSD for MMA peak area at a concentration of 1.2 µg/mL was 0.4%. The results are shown in Table 2.

**Stability of solutions**

Standard solutions at the LOQ concentration of MMA were analyzed: a freshly prepared (injected six times) and after 24 h of storage at room temperature (injected three times). RSD was 4.2% (n = 9). After 48 h of storage, a decrease of the peak area of MMA (about 50% of the initial value) was observed.

Stability of the sample solution was also confirmed. RSD of peak area of MMA in a sample solution was found to be 4.9% (n = 4) for the solution prepared immediately prior to analysis and after 24 h of storage at room temperature (each applied two times into the column). This confirms that both standards and sample solutions were stable at least 24 h at room temperature.

**Effect of filtration**

Standard solution of MMA at the LOQ concentration and the same solution filtered through different types of filter membranes such as: polyvinylidene difluoride PVDV-45/25, glass fibre GF-100/25 and polyethylene PET-45/25 were injected into the column (each applied three times).

Usage of a polyvinylidene difluoride PVDV-45/25 membrane filter resulted in an overestimation of the MMA peak area. The mean value of this peak area recorded for filtered solution represents 148.2% of the mean surface area recorded for not filtered solution. The repeatability (RSD = 13.4%) exceeds the acceptance limit RSD < 10%.

---

**Table 3. Robustness of the developed HPIC method.**

<table>
<thead>
<tr>
<th>Flow rate</th>
<th>Monomethylamine (MMA)</th>
<th>Resolution R_s as ammonium / monomethylamine</th>
<th>Resolution R_s as monomethylamine / monomethylamine</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Retention time (min)</td>
<td>Symmetry factor A_s</td>
<td></td>
</tr>
<tr>
<td>0.8 mL/min</td>
<td>5.20</td>
<td>1.24</td>
<td>1.26</td>
</tr>
<tr>
<td>1.0 mL/min</td>
<td>4.58</td>
<td>1.23</td>
<td>1.27</td>
</tr>
<tr>
<td>1.2 mL/min</td>
<td>3.98</td>
<td>1.18</td>
<td>1.21</td>
</tr>
<tr>
<td>Mobile</td>
<td>Mobile phase</td>
<td>Mobile phase</td>
<td>Mobile phase</td>
</tr>
<tr>
<td>8 mM MSA</td>
<td>4.92</td>
<td>1.26</td>
<td>1.31</td>
</tr>
<tr>
<td>10 mM MSA</td>
<td>4.50</td>
<td>1.23</td>
<td>1.27</td>
</tr>
</tbody>
</table>

---

**Table 4. Recovery of monomethylamine (MMA) from model solutions.**

<table>
<thead>
<tr>
<th>Theoretical concentration of MMA</th>
<th>Recovery [%]</th>
<th>RSD (n = 3) [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.6 µg/mL (20% of investigation range)</td>
<td>96.4</td>
<td>5.3</td>
</tr>
<tr>
<td>1.2 µg/mL (50% of investigation range)</td>
<td>91.7</td>
<td>5.6</td>
</tr>
<tr>
<td>2.0 µg/mL (80% of investigation range)</td>
<td>96.7</td>
<td>4.5</td>
</tr>
</tbody>
</table>

---

**Table 5. Results of monomethylamine (MMA) determination in nebivolol hydrochloride.**

<table>
<thead>
<tr>
<th>RSD (n = 6)</th>
<th>Reading from calibration curve</th>
<th>Multiple standard addition method</th>
</tr>
</thead>
<tbody>
<tr>
<td>Assay of monomethylamine (MMA) X ± ΔX (PU= 95%)</td>
<td>4.6 ± 0.2 ppm</td>
<td>4.5 ± 0.4 ppm</td>
</tr>
</tbody>
</table>
On the other hand, using of glass fibre GF-100/25 filters causes decrease of the peak areas of MMA to the level of noise.

RSD of peak area of monomethylamine was found to be 2.8% \((n = 6)\) for not filtered solutions and solutions filtered through the membrane filter (PET-45/25). It confirmed the lack of adverse effect of filtration when polyethylene filters are used.

**Robustness**

The effect of the mobile phase flow ratio \((\pm 0.2 \text{ mL/min})\) and composition \((\pm 2 \text{ mM MSA})\) on retention time, symmetry factor \((A_s)\) of MMA peak and resolution \((R_s)\) between peaks of monomethylamine and ammonium; monomethylamine and monoethylamine were evaluated for the standard mix solutions \((\text{approximately } 0.5 \mu \text{g/mL})\). The results are presented in Table 3.

**Accuracy**

The accuracy of the method was confirmed using aqueous solutions of nebivolol hydrochloride at a concentration of 10 mg/mL with methylamine addition: at a concentration of 0.6 \(\mu\text{g/mL}\) (about 20%), at a concentration of 1.2 \(\mu\text{g/mL}\) (about 50%) and 2.0 \(\mu\text{g/mL}\) (which is about 80% of the studied concentration range). The results are shown in Table 4.

**Content determination of MMA in nebivolol hydrochloride**

**Standard solutions**

Six aqueous solutions of MMA were prepared:
- standard solution 1 \((0.03 \mu\text{g/mL of MMA})\); LOQ
- standard solution 2 \((0.2 \mu\text{g/mL of MMA})\)
- standard solution 3 \((0.6 \mu\text{g/mL of MMA})\)
- standard solution 4 \((1.2 \mu\text{g/mL of MMA})\)
- standard solution 5 \((3.2 \mu\text{g/mL of MMA})\)
- standard solution 6 \((4.0 \mu\text{g/mL of MMA})\)

**Sample solutions**

**Unspiked sample**

Half gram of nebivolol hydrochloride was weighed and transferred to a conical flask, 25.0 mL of water was added to the sample. The mixture was mechanically shaken for 1 h and filtered through a membrane filter Chromafil PET-45/25. Then, the filtrate was used to prepare sample solutions as below:
- 5.0 mL of water was added to 5.0 mL of the filtrate

**Spiked sample**

- 5.0 mL of standard solution 4 was added to 5.0 mL of the filtrate \((0.6 \mu\text{g/mL of MMA})\)
- 5.0 mL of standard solution 5 was added to 5.0 mL of the filtrate \((1.6 \mu\text{g/mL of MMA})\)
- 5.0 mL of standard solution 6 was added to 5.0 mL of the filtrate \((2.0 \mu\text{g/mL of MMA})\)

Aliquots of 100 \(\mu\text{L}\) of the prepared standards and sample solutions were injected into the chromatographic column.

The analysis was carried out using the HPIC system described previously by reading from a calibration curve as well as the method of standard addition. For fortified samples, the concentration of methylamine was obtained with the graphical extrapolation method using the calibration curve. The results are shown in Table 5.

Examples of chromatograms of the solvent, samples of nebivolol hydrochloride and the samples containing of MMA standard additions at a concentration of 4 and 60 ppm and are shown in Figure 1.

**CONCLUSIONS**

A simple, environmentally friendly HPIC method with isocratic elution was elaborated, vali-
dated and successfully applied for the determination of monomethylamine (MMA) in an active substance – nebivolol hydrochloride.

The developed method was characterized by good precision and sufficient sensitivity for determination of monomethylamine in the investigated substance at the ppm level.

Amount of the MMA was determined by direct reading from the calibration curve and by the multiple standard addition method. Both methods showed satisfactory precision (RSD < 10%) and they can be used to determine the MMA content in the studied active substance.

This method also exhibited good selectivity for determination of MMA. A satisfactory separation of monomethylamine from other short-chain aliphatic amines, alkanolamines and metal cations were achieved (Table 1). Only monoethanolamine was co-eluted with the same retention time as the MMA.

The above-described HPIC method can be used to determine the MMA and many other short-chain aliphatic amines, alkanolamines in a variety of samples of different origin.

Use of the multiple standard addition method allows for the elimination of the potential matrix effects or an interference from other components.

The developed HPIC method can be successfully used in routine analysis and even replace the GC method commonly used in the specifications supplied by manufacturers of active substances that can by contaminated by the above-mentioned amines.

REFERENCES


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