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Uniformly sized molecularly imprinted polymer for atropine and its application to the determination of atropine and scopolamine in pharmaceutical preparations containing *Scopolia* extract

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Abstract

A uniformly sized molecularly imprinted polymer (MIP) for atropine has been prepared. The MIP was prepared using 2-(trifluoromethyl) acrylic acid and ethylene glycol dimethacrylate as a functional monomer and cross-linker, respectively, by a multi-step swelling and thermal polymerization method. The selectivity factor, which is defined as the ratio of the retention factors (k) on the molecularly imprinted and non-imprinted polymers, $k_{\text{imprinted}}/k_{\text{non-imprinted}}$, was 2.2 for atropine on the MIP. The obtained MIP was applied for the determination of tropane alkaloids (atropine and scopolamine) in a commercial gastrointestinal drug by a column-switching HPLC system, consisting of an MIP material as a pre-column, and a conventional cation-exchange analytical column. An interference peak was observed at the retention time of atropine derived from pre-column. However, since the peak area was less than 0.5% the peak area of atropine of a standard solution under the analytical conditions of this study (0.2 μg of atropine was loaded), this interference was negligible in the determination of atropine. On the other hand, no interference peak was observed at the retention time of scopolamine. Calibration curves of atropine and scopolamine showed good linearity in the range of 0.02–0.9 μg/ml ($r = 0.9999$) and 0.003–0.09 μg/ml ($r = 0.9998$), respectively. The mean recoveries of atropine and scopolamine from a placebo pharmaceutical preparation sample were 98.9 and 99.9%, respectively. The intra-day precision (measured by relative standard deviation, R.S.D. (%)) of both ingredients was less than 2.0%. The optimized column-switching system was applied successfully to the determination of atropine and scopolamine in a commercial gastrointestinal drug.

Keywords: Molecularly imprinted polymer; Atropine; Scopolamine; Scopolia extract; Column switching; Pharmaceutical analysis.

1. Introduction

Scopolia extract is an important crude drug for the treatment of gastrointestinal diseases, cardiopathy and Parkinson's disease. Scopolia extract contains tropane alkaloids, such as atropine (racemic hyoscyamine) and scopolamine, as the main ingredients. The structures of atropine and scopolamine are shown in Fig. 1.

A number of analytical methods have been reported for the determination of tropane alkaloids [1], such as thin-layer chromatography [2], gas chromatography [3,4], capillary electrophoresis [5–7] and high-performance liquid chromatography (HPLC) [8–10].

However, even when using these methods, the determination of tropane alkaloids in pharmaceutical preparations often requires several complicated sample preparation processes, such as extraction, concentration and/or derivatization. This is because the dosage level of scopolia extracts are generally very low (the total alkaloid content calculated as the free base is 0.90–1.09% of the scopolia extract

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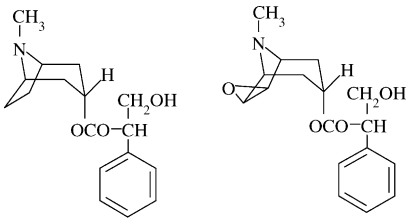

Fig. 1. Structures of atropine and scopolamine.

[11]) and impurities from large doses of other crude drugs or ingredients usually interfere chromatographically. Although these pre-treatment operation processes are essential, they are time-consuming and involve the possibility of generating experimental errors in the final determination results.

As a simple and convenient means for concentration and purification of low concentration analytes in complex matrices, solid-phase extraction (SPE) is known [12]. Particularly, SPE based on molecularly imprinted polymers (MIPs), which show high selectivity in rebinding the template (target analytes), have achieved selective enrichment and pre-treatment of target analytes in complex matrices [13]. Theodoridis et al. [14] reported the MIP for hyoscyamine prepared by a typical bulk polymerization method as off-line SPE, and they applied it to the selective extraction of scopolamine from biological samples.

When compared with an off-line mode, on-line SPE coupled to an HPLC system is advantageous for routine quality control analysis of pharmaceutical preparations, because it allows easy automation with high sample throughput and good repeatability, thus, analysis can be accomplished within a shorter time [15]. MIPs for on-line SPE have already been widely applied to biological and environmental analyses [16–18].

Therefore, our aim was to prepare an MIP for atropine suitable for on-line SPE and apply it to an on-line pre-treatment device to determine tropane alkaloids in pharmaceutical preparations produced using HPLC. To achieve this objective, we prepared a uniformly sized MIP for atropine using a multi-step swelling and polymerization method. This method allows for the facile preparation of spherical particles with a narrow particle distribution; making it suitable for as an HPLC packing material [19–23].

In this study, the retentive property of atropine and scopolamine on the MIP obtained was evaluated. Further, the MIP was applied to determination of tropane alkaloids in a commercial gastrointestinal drug using a column-switching HPLC system. As a result, simultaneous determination of atropine and scopolamine in a commercial gastrointestinal drug (Table 1) was successfully achieved by an optimized column-switching system without complicated pre-treatment. Validation of this atropine and scopolamine determination method is also described.

Table 1
Content of each active ingredient in a commercial gastrointestinal preparation

Ingredients	Content (mg/one day dose)
Scopolia extract 3 triturated powder	90
(Total alkaloids	0.29)
Sofalcone	300
Neusiline (magnesium aluminometa silicate)	900
Sodium hydrogen carbonate	600
Other compounds	1010

2. Experiment

2.1. Materials

Ethylene glycol dimethacrylate (EDMA) and 2-(trifluoromethyl)acrylic acid (TFMAA) [24] were purchased from Tokyo Chemical Industry (Tokyo, Japan). These monomers were purified by general distillation techniques in vacuo to remove the polymerization inhibitor. Also purchased were 2,2′-Azobis (2,4-dimethylvaleronitrile) from Wako (Tokyo, Japan), and atropine, from Sigma-Aldrich Japan (Tokyo, Japan). The reference standards of scopolamine hydrobromide and atropine sulfate were purchased from the Japanese Pharmacopoeia (Tokyo, Japan). Scopolia extract 3 triturated powder raw material was purchased from Alps Pharmaceutical Ind. Co. Ltd. (Gifu, Japan). HPLC-grade acetonitrile was also obtained from Wako. A CAPCELL PAK SCX column (15 mm × 4.6 mm i.d.) was obtained from Shiseido (Tokyo, Japan). Other reagents and solvents of analytical–reagent grade were used without further purification. Water purified with a Milli-RO 60 water purification system (Nihon Millipore Tokyo, Japan) was used to prepare the eluent and the sample solution.

2.2. Preparation of uniformly sized MIP for atropine; multi-step swelling and polymerization method

Preparation of the uniformly sized MIP for atropine as well as the non-imprinted polymer (NIP) was carried out as described in a previous paper [19], by a multi-step swelling and polymerization method. The molar amounts of atropine, TFMAA and EDMA as shown in Table 2 were used as a template, functional monomer and cross-linker, respectively. The

Table 2
Molar amounts of template (atropine), functional monomer (TFMAA) and cross-linker (EDMA) used for the preparation of MIP for atropine and NIP

	Amount (mmol)		
	Template	TFMAA	EDMA
MIP	2	7	25
NIP	0	7	25

prepared polymers were packed into stainless steel columns (50 mm × 4.6 mm i.d. and 10 mm × 4.6 mm i.d.) by a slurry packing technique using methanol-2-propanol (2:1, v/v) as the slurry and packing solvents.

2.3. Evaluation of MIP for atropine

The HPLC system used was composed of an LC-10AD pump, SPD-10AV spectrophotometer, CTO-10AC column oven, and C-R7A plus integrator (Shimadzu, Kyoto, Japan). The flow rate was maintained at 0.5 ml min^{-1}. Detection was performed at 210 nm. Retention factors were calculated from the equation $k = (t_R - t_0)/t_0$, where t_R and t_0 are retention times of retained and unretained solutes, respectively. The retention time of unretained solute, t_0, was measured by injecting acetone. The selectivity factor is calculated from the equation $S = k_{imprinted}/k_{non\text{-}imprinted}$, where $k_{imprinted}$ and $k_{non\text{-}imprinted}$ are retention factors on the MIP and NIP, respectively. All separation were carried out at 30 °C. The eluent were prepared by using phosphoric acid, potassium dihydrogenphosphate, dipotassium hydrogenphosphate and acetonitrile. The eluent used is specified in the legends of figures.

2.4. Application of MIP for determination of atropine and scopolamine

In addition to the HPLC system described above, an LC-10AD pump and a six-port switching valve (FCV-12AH,Shimadzu, Kyoto, Japan) were used. Fig. 2 shows a schematic diagram of column-switching HPLC system employed in this study.

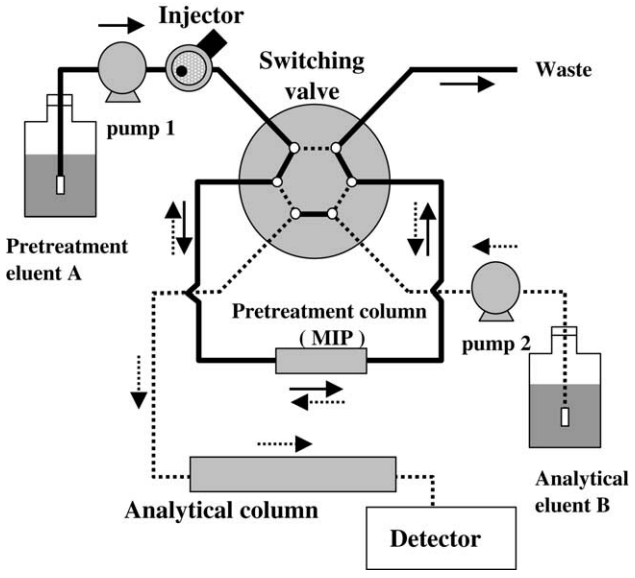

Fig. 2. A schematic diagram of the column-switching HPLC system used in this study. Solid line: pre-treatment step, dotted line: separation step.

The pre-column packed with MIP (10 mm × 4.0 mm i.d) was equilibirated with potassium dihydrogenphosphate and dipotassium hydrogenphosphate buffer (pH 6.0; 25 mM)—acetonitrile (80:20, v/v) (eluent A), and 400 μl of a sample solution was loaded. The pre-column was washed for 5 or 10 min with the eluent A at a flow rate of 0.5 ml min^{-1} to remove other compounds. Then, the six-port switching valve was actuated, and atropine and scopolamine retained on the pre-column was swept to the analytical column (CAPCELL PAK SCX UG-80, 15 cm × 4.6 mm i.d.) in the back-flush mode by potassium dihydrogenphosphate and phosphoric acid buffer (pH 3.0; 50 mM)—acetonitrile (80:20, v/v) (eluent B) at the flow rate of 1.0 ml min^{-1}. The pre-column and analytical columns were operated at 40 °C using the column oven. Detection was performed at 210 nm. The pre-column was switched back after 5 min and equilibrated with eluent A. Atropine and scopolamine were separated on the analytical column with eluent B.

2.5. Procedure for preparation of sample and standard solutions

To determine the ingredients in a commercial gastrointestinal drug (Table 1), a representative number of tablets (usually 20) were accurately weighed and finely powdered. An adequate amount of a sample (equivalent to about 7 mg of scopolia extract 3 triturated powder) was weighed accurately and 20 ml volumes of 0.5 mol l^{-1} hydrochloric acid solution were added accurately. The mixture was sonicated for 15 min and centrifuged at 3500 rpm for 10 min. Five millilitre of the supernatant was accurately pipetted and sodium hydroxide solution was added to adjust the pH to 6.0, then potassium dihydrogenphosphate and dipotassium hydrogenphosphate buffer (pH 6.0; 50 mM) was added to produce exactly 10 ml (sample solution). The final atropine and scopolamine concentration of sample solution was about 0.5 and 0.05 μg ml^{-1}, respectively. The reference standards of scopolamine hydrobromide and atropine sulfate were weighed and diluted to concentrations similar to those in the prepared sample solution (standard solution). These solutions were analyzed in 400 μl volumes under the HPLC conditions described above.

3. Result and discussion

3.1. Preparation and Evaluation of TFMAA-EDMA polymers

Approximately 5 g of MIP and NIP were obtained, under the conditions given in Table 2. The optical micrographs of MIP and NIP were shown in Fig. 3. Good size uniformity was obtained and there was no difference in the shape of the particles of both polymers. The average particle diameter of these polymers was about 7 μm.

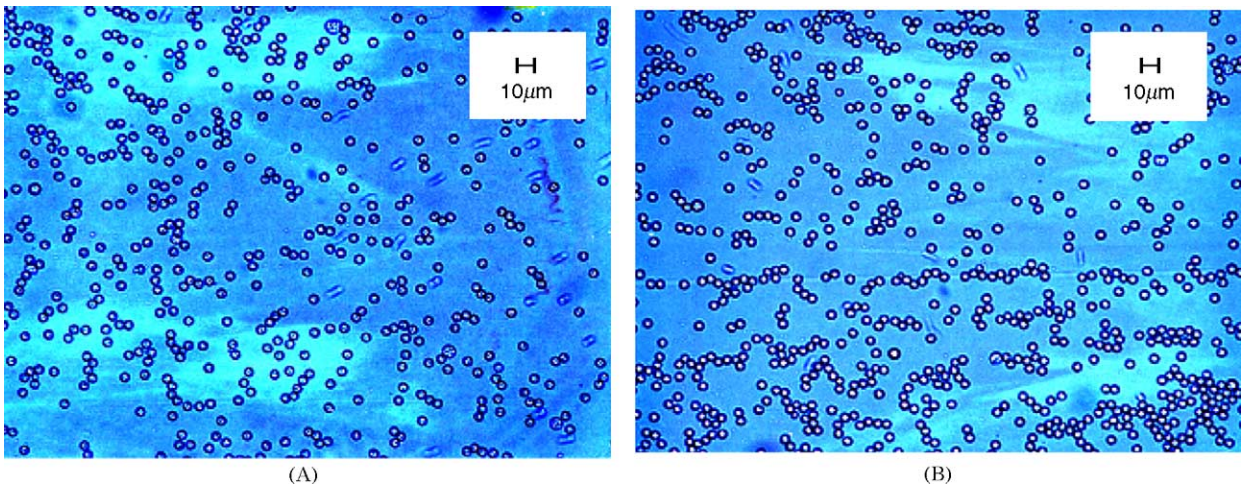

Fig. 3. Optical micrographs of atropine-imprinted TFMAA-EDMA (A) and non-imprinted TFMAA-EDMA (B) particles prepared by multi-step swelling and polymerization method (200x magnification).

3.2. Selectivity of the atropine-imprinted TFMAA-EDMA polymer

Selectivity of the atropine-imprinted TFMAA-EDMA polymer toward atropine and scopolamine was examined. Fig. 4 shows chromatograms obtained with a 0.4 μg injection of atropine standard solution on atropine-imprinted TFMAA-EDMA (MIP) and non-imprinted TFMAA-EDMA (NIP) columns. The retention factors for atropine on the MIP and NIP were 3.7 and 1.7, respectively. The selectivity factor for atropine was 2.2. Further, the selectivity factor for scopolamine was 1.5 (data not shown). These results confirmed that the obtained MIP has selective retention properties for atropine and for its structurally related compounds by molecular imprinting effect. Since the retention ability of MIP for atropine and scopolamine was higher than that of NIP, MIP was used for the following experiments.

3.3. Retention properties of atropine and scopolamine on the atropine-imprinted TFMAA-EDMA polymer

Fig. 5 shows the effects of eluent pH on the retention properties of atropine and scopolamine on MIP column. The maximum retention factors of atropine and scopolamine were observed at eluent pH values of 7.0 and 6.0, respectively. The retention of atropine and scopolamine is thought to be caused by ionic interactions between positively charged amino groups of these basic compounds and negatively charged TFMAA-EDMA polymer in the neutral pH ranges. The

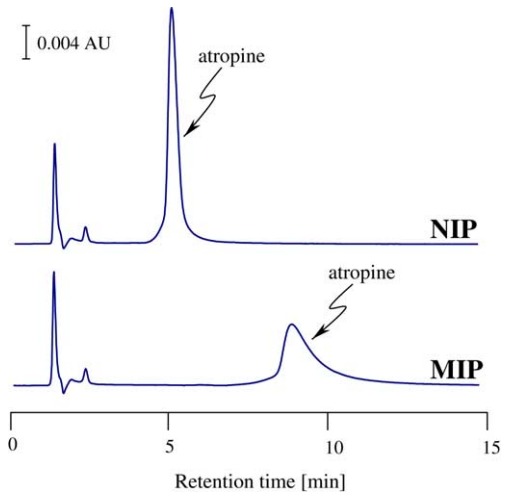

Fig. 4. Chromatograms of atropine on atropine-imprinted TFMAA-EDMA (MIP) and non-imprinted TFMAA-EDMA (NIP). HPLC conditions: column size, 50 mm ± 4.6 mm i.d.; eluent, potassium dihydrogenphosphate and phosphoric acid (pH 3.0; 10 mM)—acetonitrile (50:50, v/v); column temperature, 30 °C; detection, 210 nm; flow rate, 0.5 ml min^{-1}; amount loaded, 0.4 μg (atropine).

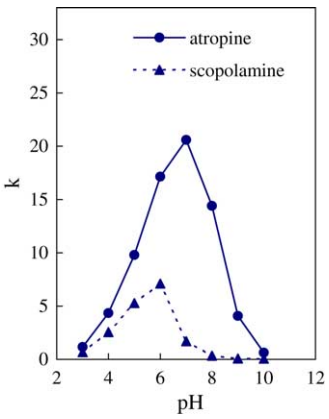

Fig. 5. Effect of eluent pH on the retention properties of atropine and scopolamine on atropine-imprinted TFMAA-EDMA (MIP). HPLC conditions: column size, 50 mm ± 4.6 mm i.d.; eluent, potassium dihydrogenphosphate and dipotassium hydrogenphosphate buffer (phosphoric acid or potassium hydroxide were added to adjust pH if necessary) (50 mM)—acetonitrile (50:50, v/v); column temperature, 30 °C; detection, 210 nm; flow rate, 0.5 ml min^{-1}; amount loaded, 0.4 μg (atropine and scopolamine).

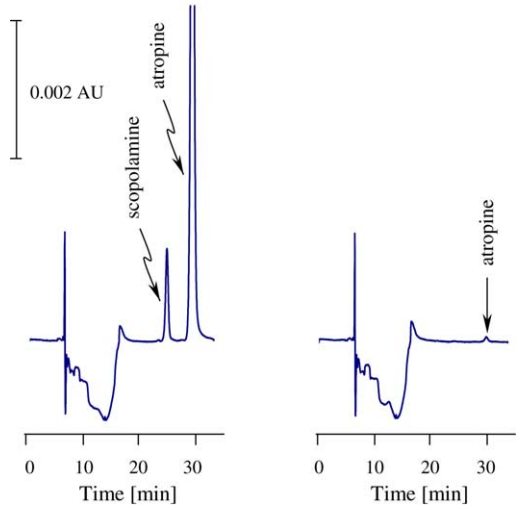

Fig. 6. Chromatograms of standard solution (A) and water (B) using column-switching techniques. HPLC conditions are described in Section 2.4.

average pK_a value of the TFMAA-EDMA polymer is below 9 [25],while the pK_a values of atropine and scopolamine are 9.7 and 7.6 [5],respectively. Using a neutral eluent, atropine and scopolamine (analytes) were adsorbed onto the MIP column. On the other hand, using an acidic or an alkaline eluent, the retention of analytes were decreased by protonation of TFMAA-EDMA polymers at an acidic eluent pH or deprotonation of the amino groups of analytes at an alkaline eluent pH. Therefore, optimal eluents selected were potassium dihydrogenphosphate and dipotassium hydrogenphosphate buffer (pH 6.0; 25 mM) – acetonitrile (80:20, v/v) for pre-treatment, and potassium dihydrogenphosphate and phosphoric acid buffer (pH 3.0; 50 mM) – acetonitrile (80:20, v/v) for analysis. Since atropine and scopolamine were easy to hydrolyze in alkaline solutions, an alkaline mobile phase was not chosen for quantitative analysis.

3.4. Application of MIP for the determination of atropine and scopolamine using a column-switching procedure

The obtained MIP was applied to the determination of atropine and scopolamine in a pharmaceutical preparation by a column switching technique. Fig. 6 shows chromatograms obtained with 400 μl injections of standard solution and water using an MIP column and conventional cation-exchange column as a pre-column and analytical column, respectively. A peak at 30 min appeared with no injection of atropine. This result suggests leakage of the imprint species from the pre-column. It has been reported that even thorough washing of the imprinted materials results in appearance of a peak corresponding to the imprint species [17]. However, since the peak area was less than 0.5% of that of atropine of the standard solution, this interference was negligible under the analytical conditions in this study; a loading of 0.2 μg of atropine. Further, no interference peak was observed at the retention time of scopolamine.

3.5. Validation data for determination of atropine and scopolamine in a pharmaceutical preparation

3.5.1. Linearity

The quantitation linearity of atropine and scopolamine in standard solution was examined. For each ingredient, the relationship between peak area and concentration was calculated. In each case, straight regression lines with a correlation coefficient (r) above 0.999 were obtained. The intercept values did not significantly vary from zero (95%). The detection limits estimated as a peak with a signal-to-noise ratio of 3, and quantitation limits as a peak with a signal-to-noise ratio of 10 of atropine and scopolamine with a 400 μl injection volume are shown in Table 3.

3.5.2. Specificity

Fig. 7 shows the chromatograms of (A) a standard solution, (B) a sample solution of a commercial gastrointestinal drug, and (C) a placebo sample solution of a commercial gastrointestinal drug. The peaks of atropine and scopolamine were clearly separated from other components in the commercial gastrointestinal drug.

An interference peak was observed at the retention time of atropine derived from pre-column. However, the peak area was less than 0.5% of that of atropine of the standard solution. This interference was negligible under the analytical conditions of this study (0.2 μg of atropine was loaded). Further, no interference peak was observed at the retention time of scopolamine.

On the other hand, Fig. 8 shows the chromatogram obtained when injecting the sample solution directly to the analytical column. In this case, large amounts of interfering compounds are detected. Particularly, the peak of scopolamine was completely hidden by an interference peak from sample matrices; making it difficult to determine scopolamine. These results indicate that the MIP can be favorably used for effective clean up of this sort of samples.

Table 3
Results of linearity study

Active ingredient	Concentration range (μg ml^{-1})	r	Intercept	Slope	LOD (μg ml^{-1})	LOQ (μg ml^{-1})
Atropine	0.02–0.9	0.9999	−1200	485000	0.008	0.024
Scopolamine	0.003–0.09	0.9998	−380	392900	0.001	0.004

r, Correlation coefficient; LOD, limit of detection; LOQ, limit of quantitation.

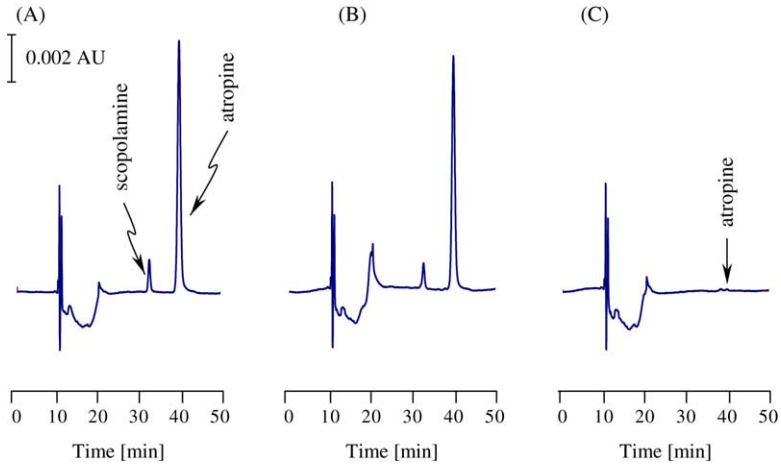

Fig. 7. Chromatograms of standard solution (A), sample solution (B) and control sample solution (C) using a column-switching HPLC system. HPLC conditions are described in Section 2.4.

3.5.3. Recovery and precision

Recovery was assessed over the entire concentration range (75, 100 and 125%) by analyzing placebos spiked with 3 triturated powder raw material solution at three concentration levels. The solutions were replicated three times each, and the amounts determined were compared with theoretical amounts. The mean recoveries of atropine and scopolamine from a placebo pharmaceutical preparation sample were 98.9% and 99.9% respectively (Table 4). Adequate results for recovery were obtained for atropine and scopolamine.

Table 4
Recovery and precision of atropine and scopolamine

Active ingredient	Added ($\mu g\,ml^{-1}$)	Recovery ± R.S.D. (%, $n = 3$)	Average (%, $n = 9$)
Atropine	0.383	98.8 ± 0.8	
	0.510	99.1 ± 0.4	98.9
	0.638	98.8 ± 0.6	(R.S.D. = 0.5%)
Scopolamine	0.0353	98.9 ± 1.5	
	0.0471	100.6 ± 1.6	99.9
	0.0589	100.1 ± 1.6	(R.S.D. = 1.5%)

R.S.D., Relative standard deviation.

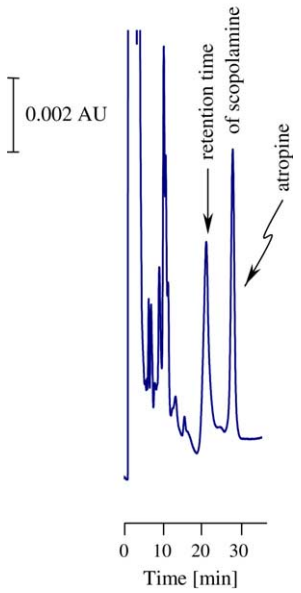

Fig. 8. Chromatogram of the sample solution that has not been subjected to MIP clean up. HPLC conditions: analytical column, CAPCELL PAK SCX (150 mm × 4.6 mm i.d); eluent, potassium dihydrogenphosphate and phosphoric acid buffer (pH 3.0; 50 mM)—acetonitrile (80: 20, v/v) at 1.0 ml min^{-1}; column temperature, 40 °C; detection, UV absorbance at 210 nm; injection volume, 400 μl.

Precision was determined by measuring ($n = 3$) each active ingredient in spiked placebos at the three concentration levels. The intra-day precision (measured by relative standard deviation, R.S.D. (%)) of both ingredients was less than 2%. Atropine and scopolamine both showed good precision.

3.6. Quantitative analysis of atropine and scopolamine in a commercial gastrointestinal drug

Results of the determination of atropine and scopolamine in a commercial gastrointestinal drug (Table 1) performed under the conditions shown in Fig. 7 are given in Table 5.

Table 5
Determination of atropine, scopolamine and total alkaloids contents in a gastrointestinal preparation

Method	Content (mg/one day dose) (R.S.D. (%), $n = 4$)		
	Atropine	Scopolamine	Total alkaloids
This study	0.263 (1.7)	0.027 (2.2)	0.290 (1.7)
Modified JP14	0.270 (2.3)	0.022 (1.2)	0.291 (2.1)

R.S.D., Relative standard deviation. Calculated amount of total alkaloids in the gastrointestinal drug is 0.29 mg/one day dose.

On the whole, good agreement was obtained between the test method of this study and the Japanese Pharmacopoeia, 14th ed. (JP 14) method [11]. In order to remove interference derived from the commercial gastrointestinal drug, the extraction procedure of JP 14 method was slightly modified. The modified JP 14 method requires some manual procedures: repetitive partitioning, transference from glassware to others, and evaporation. Although these procedures are essential, they are complicated, laborious and difficult to complete without loss of actual sample amount. On the other hand, the proposed method enables accurate determination of presence of atropine and scopolamine in a commercial gastrointestinal drug without such complicated pre-treatment operations.

4. Conclusion

A uniformly sized MIP for atropine has been prepared. The obtained MIP was applied to an on-line pre-treatment column to determine tropane alkaloids (atropine and scopolamine) in a commercial gastrointestinal drug using a column-switching HPLC system. An interference peak observed at the retention time of atropine derived from pre-column was negligible in the determination of atropine because the area of the peak was less than 0.5% of that of atropine of a standard solution, based on the analytical conditions of this study. The proposed method was accurate and reproducible on the basis of the result of recovery (98–99%) and small coefficient of variations of intra-day assay (less than 2%). This method will applicable to the determination of atropine and scopolamine in many other pharmaceutical preparations without complicated pre-treatment, and will be useful for routine quality control analysis as a laborsaving method.

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