Ion Chromatographic Determination of Residual Phase Transfer Catalyst in Active Pharmaceutical Ingredient

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Abstract

A new ion chromatography method with non-suppressed conductivity detection has been developed for the quantification of residual phase transfer catalyst–tetrabutylammonium bromide (TBAB) in an active pharmaceutical drug, Levetiracetam. Separation conditions are optimized to get a clear separation of TBAB from drug impurities using a Metrosep Cation C2–150 column. Conditions are also optimized to separate tetramethylammonium bromide, tetraethylammonium bromide, and tetrapropylammonium bromide, which are also used as phase transfer catalysts in several syntheses. Method performance was checked for selectivity, linearity, limit of quantification, limit of detection, accuracy, and precision. The method has superior performance with linearity $r^2 \geq 0.9999$, recovery from 94.7% to 96.5%, precision ≤ 0.74%. In-line preconcentration is used to achieve limits of detection and quantification of 39 ng and 118 ng of TBAB, which corresponds to 1.56 and 4.72 µg/g of TBAB with respect to sample weight. The proposed method can be used for routine quality assurance analysis in the pharmaceutical industry.

Introduction

Levetiracetam is an antiepileptic drug indicated as adjunctive treatment for partial onset seizures in adults with epilepsy. The chemical name for levetiracetam, a single enantiomer, is (S)-a-ethyl-2-oxo-1-pyrrolidineacetamide. Epilepsy is a common chronic neurological disorder that is characterized by recurrent unprovoked seizures. Along with other anticonvulsants like gabapentin, levetiracetam is also sometimes used to treat neuropathic pain. Levetiracetam is marketed under the trade name Keppra. The pKa value of this drug is 4.95. Tetrabutylammonium bromide (TBAB) is used as phase transfer catalyst in the product synthesis.

Determination of low-molecular weight amines is either carried out by gas chromatography (GC) or by high-performance liquid chromatography (HPLC). Few other studies indicate the possibility of separation by capillary electrophoresis with UV detection. However, aliphatic amines have poor detectability, owing to the absence of any chromophoric groups in the molecule and hence the chemical derivatization is an ideal choice to improve the specificity and sensitivity of the analysis by HPLC.

Among numerous HPLC methods that have been developed for the separation and determination of amino compounds, some are very well adapted for application to particular compounds, but more recent ones involve separation after precolumn derivatization (1–7).

Determination of aliphatic amines by GC creates additional challenges due to their high aqueous solubility, volatility, polarity, and basic character. As the molecular mass of the amine decreases, the relative effect of the amine group increases, which results in stronger sorption to polar stationary phases. Various derivatizing agents are used for aliphatic amine quantification by GC (8–12). However, derivatization methods are time-consuming. In addition, the selectivity of amine derivatives cannot be guaranteed for the analysis of complex mixtures, such as pharmaceutical drugs.

A method by GC (13) is reported for TBAB quantification, which involves pyrolysis of TBAB to convert to *n*-butyl bromide and tertiary butylamine, followed by GC separation and quantification. But this method is not suitable for trace level determination.

Aliphatic amines can be separated by cation chromatography on suitable stationary phases and detected via suppressed or non-suppressed conductivity detection (14–17). Several references are available for the determination of low molecular weight aliphatic amines in pharmaceutical matrices (18–21), but so far no ion chromatography (IC) method has been reported for the determination of TBAB.

The present work deals with the determination of trace level TBAB in the pharmaceutical matrix. The scope of this work is to develop an IC method to determine TBAB with a sensitivity to quantify less than 10 µg/gm of TBAB with respect to the drug weight.

Experimental

Instrument and column

A Professional IC 881 and 858 Professional sample processor with built in injector and peristaltic pump from Metrohm (Herisau, Switzerland) was used for sample handling. Con-

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trolling and data acquisition was done through MagICNet software. Silica-based Metrosep cation C2–150 column and polymethacrylate-based Metrosep cation C3–250 columns, having identical poly butadiene maleic acid functional group, were used for the initial chromatography study. A very high capacity cation preconcentrator column (VHPCC) was used for TBAB preconcentration. The preconcentrator column had a particle size of 35 µm. The higher particle size offers the flexibility that a low pressure peristaltic pump of sample processor can be used for sample preconcentration. Also, the preconcentrator column has an operating pH range of 1 to 14 so that samples with a wide range of pH can be analyzed.

Chemicals and reagents

Suprapure nitric acid from Fluka and acetonitrile from Merck India were used for mobile phase preparation. Tetramethylammonium bromide (TMAB), tetraethylammonium bromide (TEAB), tetrapropylammonium bromide (TPAB), and TBAB standards were purchased from Fluka (Germany). Three different batches of levetiracetam and the drug impurities S-amino butyric acid and leveacid mixture were received from a pharmaceutical company in Baroda, Gujarat, India. All solutions were prepared using deionized water (> 18 MΩ) purified by a Milli-Q Gradient system (Millipore, Billerica, MA).

Eluent composition of 7.5 mM nitric acid with 35% acetonitrile in ultra-pure water (resistivity > 18 MΩ), was prepared using suprapure nitric acid from Fluka and analytical grade acetonitrile from Merck India.

One hundred twenty-five milligrams of the sample were accurately weighed to a 5-mL volumetric flask and dissolved with 3 mL of 7.5 mmol/L nitric acid, sonicated for complete dissolution, and made up to the volume with nitric acid. The sample solution was filtered using a 0.2 micron nylon filter and the filtrate was used for further analysis with IC by direct injection.

Fifty milligrams of the sample was weighed and diluted to 10 mL with 7.5 mmol/L nitric acid for in-line preconcentration method. To preconcentrate 5 mL of the sample solution, a higher sample volume was necessary to be kept in the sample processor. Hence, the sample weight dilution was modified for the in-line preconcentration method.

Results and Discussion

Separation optimization

The elution properties of TBAB and other ammonium bromide compounds (TEAB, TMAB, and TPAB) were examined on both silica and polymer-based columns. The elution was only achieved with a silica-based column.

The amine group imparts high dipole moment on amine molecules. This dipole moment is responsible for the strong interaction with silane groups of the column. As amines have both ionic and lipophilic character, their retention depends on two distinct mechanisms: ion exchange and lipophilic interaction with the alkyl chain bearing the ionic sites. Therefore, the mobile phase is made up in such a way as to use both mechanisms to control the elution. The eluent's pH was from 1.8 to 1.9. Regarding the ion-exchange mechanism, the strength of a simple acidic solution, even at low pH, is not sufficient to elute amines with a suitable efficiency. The addition of organic modifier was necessary for their elution.

Organic solvents alter the dielectric constant of the eluent, which directly influences the ionization constant of the carboxylate functional groups on the stationary phase and thus resulting in a reduction of column capacity. The additions of these modifiers have proven especially useful for strongly retained amines. The effect of the proportion of acetonitrile in the mobile phase on the retention of these compounds is shown in Figure 1. Separation of the quaternary ammonium salts was achieved with 20% acetonitrile and is shown in Figure 2. Thirty-five percent acetonitrile resulted in close elution of all four compounds.

Specificity

Method selectivity was checked by injecting TBAB, mixed cation containing sodium, ammonium, potassium, calcium and magnesium, mixed amines containing monomethyl

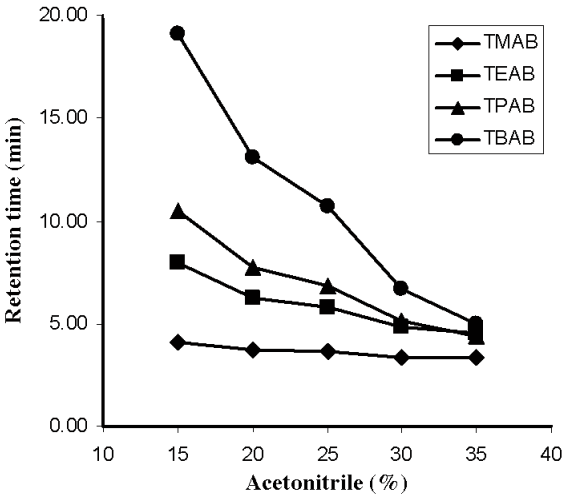

Figure 1. Acetonitrile influence on quaternary alkyl ammonium bromide's retention.

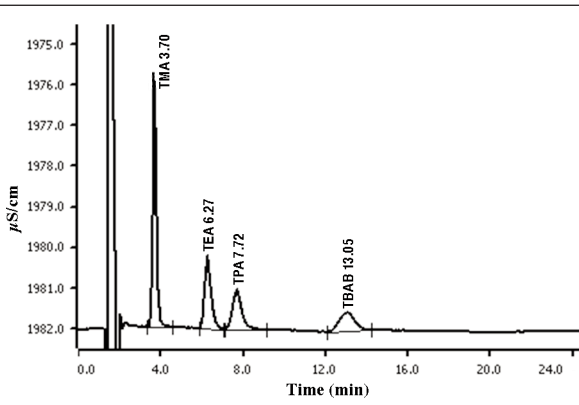

Figure 2. Separation of quaternary alkyl ammonium bromide. Column: Metrosep C2–150; eluent: 7.5 mmol/L nitric acid/20% acetonitrile; flow-rate: 1.0 mL/min.

amine, dimethyl amine, trimethylamine, diethylamine, triethylamine, ethanolamine, triethanolamine, dibutyl amine and tributyl amine, drug impurities S-amino butyric acid and leveacid mixture, the drug, and drug spiked with TBAB. The drug impurities eluted in the void volume. All the standard cations and amines eluted within four minutes' retention time. Tributylamine eluted at 4.46 min and with a clear separation from TBAB eluted at 6.78 min. Separation of TBAB from the closely eluting amine impurities is shown in Figure 3.

The clear separation of TBAB from all other product impurities and common cations and aliphatic amines proves that this chromatographic condition is suitable for the separation and quantification of TBAB in this drug matrix.

Direct injection method

In this method, 100 µL of the standard and sample solutions were injected directly to the IC system, and the method parameters were carried out, like system precision, linearity, and spiking study to check the accuracy. System precision was checked by injecting 1.0 µg/mL of TBAB standard six times, and the resulting relative standard deviation (RSD) for area and retention time was calculated as 1.85% and 0.13%, respectively. Linearity was checked by injecting TBAB of concentrations 1, 2, 10, 25, 30, and 40 µg/mL, and the relationship between peak response and concentration was found to be linear with the coefficient of determination (r^2) of 0.99988 and the RSD was 1.625%. Based on signal-to-noise ratio, the limit of quantification (LOQ) and limit of detection (LOD) were calculated as 1 and 0.2 µg/mL of TBAB, respectively, which corresponds to 40 µg/g and to 8 µg/g of TBAB with respect to the sample weight. To check the accuracy, samples were spiked with TBAB to get 1 µg/mL, 10.0 µg/mL, and a recovery study was carried out. Recovery ranging from 97% to 99% was obtained with an overall RSD value of 2.68%. The method performance indicates that the direct injection method is suitable for sensitivity up to 40 µg/g of TBAB, with respect to the sample weight, and can be used for screening TBAB content in pharmaceutical products.

In-line preconcentration method

To achieve the required sensitivity of 10 µg/g of TBAB with respect to the sample weight, one can increase the sample weight to the desired value. However, as the drug is an expensive one, higher sample quantity will not be available for routine applications. Hence, an in-line preconcentration technique was used. In this set up, the loop is replaced with the VHPCC.

The peristaltic pump built into the autosampler was used for transferring the filtered sample solution from the sample vial into the preconcentration column. The speed of the peristaltic pump can be optimized depending on the need. It is recommended to operate the system at 0.5 mL/min for a better reproducibility because a higher flow-rate reduces the column capacity somewhat, as the analyte ions have less time to interact with the resin surface. The pump was switched on for 10 min so that ~ 5 mL of the standard/sample solution was preconcentrated. After 10 min of standard/sample pumping, the sample processor moved to a special beaker with ultra-pure water and pumped ultra-pure water for 2 min to transfer the standard/samples remaining in the line to the preconcentrator column. Then the preconcentrated standard or sample was injected to the IC system. Three min after the sample injection, the injection was switched back to fill position and the next standard or sample preconcentration was carried out. This way, while a run was going on, the next standard or sample was preconcentrated to reduce the total analysis time per sample.

Breakthrough volume for VHPCC. The breakthrough volume of an analyte ion is that volume of sample which causes the ion to elute from the concentrator column.

In this case, because the sample is soluble in water but has no interaction with the preconcentration column, 10 µg/mL of TBAB, the highest concentration of TBAB that is expected in the sample, was used as the eluent and the break through volume is calculated as 22 mL. That means the VHPCC column has the capacity to preconcentrate ~ 220 µg of TBAB. However, practically the breakthrough volume will be less than the one determined, as the breakthrough volume is dependent on the volume of sample loaded, the pH of the sample, ionic strength of the sample apart from the rate at which the sample is loaded, and the amount and capacity of the resin in the preconcentrator column.

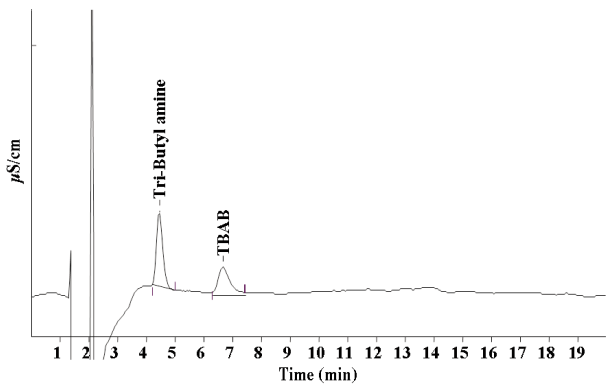

Figure 3. Selectivity separation of TBAB from drug impurities, common cations, and amines.

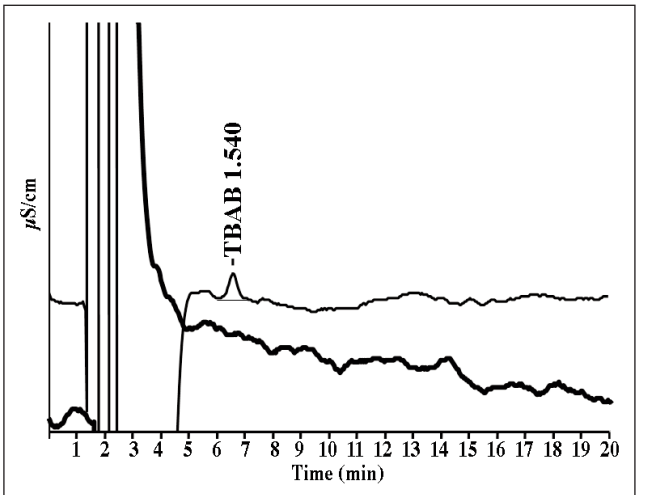

Figure 4. Sensitivity comparison sample analysis by direct injection and in-line preconcentration methods.

Linearity. TBAB concentration from 0.05, 0.1, 0.2, 0.4, 0.6, and 2.0 µg/mL were prepared from TBAB stock solution. Approximately 5 mL of the standard solution were preconcentrated and injected to the IC system. Correlation coefficient of 0.9999 with a % RSD of 2.40% was obtained. Slope value was 0.4104 ± 0.001738 with a *Y*-intercept value of –2.127 ± 1.518. The residual standard deviation was S*y*/*x* = 4.949, and this value was used for LOD and LOQ calculation.

LOD and LOQ. LOD and LOQ were predicted based on the linearity data using the following formulas.

LOD (ng) = 3.3 × Residual standard deviation (S*y*/*x*)/Slope.
LOQ (ng) = 10 × Residual standard deviation (S*y*/*x*)/Slope.

The LOD and LOQ are calculated as 38.996 and 118.19 ng of TBAB, respectively, which would correspond to 1.56 and 4.72 µg/gm of TBAB with respect to the drug weight. The detection and quantification limits can be further improved by preconcentrating higher standard and sample volume and also by taking higher sample weight. The LOD and LOQ achieved by the preconcentration technique were at least 20 times more sensitive than the direct injection technique.

Table I. TBAB Spiking and Recovery Data

TBAB in Sample (ng)	Spiked TBAB (ng)	Experimental (ng)	Recovery (%)	Average Recovery (%)	Precision RSD (%)
46.00	100.00	140.00	95.890	96.35	0.41
		141.00	96.575		
		141.00	96.575		
46.00	200.00	233.00	94.715	95.80	0.98
		237.00	96.341		
		237.00	96.341		
Overall Recovery			96.073		
Overall Precision			0.740		

Table II. Validation Summary

Validation Parameter	Acceptance Criteria	Validation Result	Result
Selectivity	Baseline separation of TBAB from tributylamine	RRT < 0.67	+
LOD	$S_{y/x}$ × 3.3/Slope	39 ng; 1.56 µg/gm	+
LOQ	$S_{y/x}$ × 10/Slope; RSD ≤ 10.0%	118 ng; 4.72 µg/gm; RSD = 0.69%	+
Linearity	r^2 ≥ 0.9980; RSD ≤ 20.0%	r^2 = 0.999; RSD = 2.40%	+
Accuracy	Spike recovery: 80…120%	98.2…103.2%	+
Precision	RSD of peak area and retention time ≤ 15%	RSD < 0.94%	+
Sample Stability	≥ 12 h at room temperature	36 h	+

Sample analysis. Five milliliters of the filtered sample solution were preconcentrated and injected into the IC system, and the TBAB content was quantified. A merged chromatogram of the sample with and without preconcentration is shown in Figure 4. In the sample, 46 ng of TBAB was detected, which is equivalent to 1.54 µg/g of TBAB with respect to the sample weight. This clearly indicates the importance of in-line preconcentration method to improve the TBAB detection in this matrix.

Accuracy and precision. To check the accuracy of the method, a spiking and recovery study was carried out with the sample. Fifty milligrams of the sample were weighed accurately and spiked with TBAB stock standard solution to get resulting concentrations of 100 and 200 ng/mL of TBAB in sample solution, and diluted to a 10-mL mark with 7.5 mmol/L nitric acid. Five milliliters of the filtered sample solution were preconcentrated and injected into the IC system. Details of the accuracy and precision data are provided in the Table I. The spiking and recovery study results indicate that there is no matrix influence, and this method is suitable for routine determination of TBAB in this sample matrix.

Sample solution stability. Standard and sample solution stability was checked at room temperature with 100 ng/mL TBAB and sample solution spiked with 100 ng/mL TBAB and both were stable at least for 36 h in sample processor vial at room temperature. The validation summary is provided in Table II.

Conclusion

A simple, direct, and rapid IC method is described for the determination of trace level phase transfer catalyst using cation-exchange non-suppressed conductivity detection. The in-line preconcentration technique helps to achieve the desired sensitivity and limit of quantification. The method has been characterized with respect to linearity, selectivity, precision, and accuracy/recovery, and is suitable for regular quality control lab for monitoring the TBAB concentration in levetiracetam.

Acknowledgment

The authors, N. Harihara Subramanian and P. Manigandan, greatly acknowledge the support provided by Metrohm India Limited, Chennai.

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Manuscript received October 23, 2008;
Revision received December 31, 2008.