Fast and Simple Determination of 3-Aminopiperidine without Derivatization Using High Performance Liquid Chromatography–Charged Aerosol Detector with an Ion-Exchange/Reversed-Phase Mixed-mode Column

Shubo DONG,* Zhengyu YAN,*† Hanyue YANG, and Zhen LONG*****

**China Pharmaceutical University, Longmian Road 639, Jiangning District, Nanjing 210000, Jiangsu, P. R. China*
***Jiangsu Deyuan Pharmaceutical Co., Ltd, Jinqiao Road 21, Lianyungang 222002, Jiangsu, P. R. China*
****Thermo Fisher Scientific Co., Ltd, Andingmen Road 28, Beijing 100000, P. R. China*

A sensitive non-derivatization method for the determination of the highly polar compound 3-aminopiperidine was developed using a mixed-mode column combined with a charged aerosol detector (CAD). Chromatographic conditions, including the type of detector, separation mode, and mobile phase composition, were optimized to achieve high sensitivity towards and sufficient retention of 3-aminopiperidine. Compared to the precolumn derivatization UV method, the current method showed higher recovery and greater simplicify. High sensitivity (LOQ <2.73 μg mL^{-1}) and good precision (RSD of peak area <2%) were also observed in the current method. Furthermore, the parameters such as buffer solution and column bleed that affected the sensitivity of the CAD were investigated. Finally, the current method was applied for the determination of 3-aminopiperidine in linagliptin samples. This is a new non-derivative for the determination of 3-aminopiperidine, and constitutes a novel application of the CAD for the quantitative analysis of highly polar basic compounds.

Keywords 3-Aminopiperidine, non-derivatization, charged aerosol detector, linagliptin

(Received August 10, 2016; Accepted October 6, 2016; Published March 10, 2017)

Introduction

Highly polar basic compounds are of great significance in the fields of life sciences and medicine, because many bioactive compounds such as neurotransmitters, metabolites of pharmaceuticals, and intermediates of drugs exhibit high polarity.[1] Moreover, some highly polar basic compounds serve as starting materials for medicines. For example, 3-aminopiperidine is an important starting material in the synthesis of DPP-4 inhibitor drugs such as alogliptin, trelagliptin, and linagliptin.[2]

However, the determination of highly polar basic compounds is not easy.[3,4] Ion chromatography (IC) provides sufficient retention for highly polar basic compounds such as amines,[5] but offers limited scope in the determination of highly polar impurities in hydrophobic drugs, because it is difficult to elute hydrophobic drugs from IC columns. Therefore, complex pretreatments (on-line or off-line) are necessary to remove hydrophobic drugs before the sample can be analyzed by IC methods. For example, for the determination of azides in irbesartan samples, Chinese Pharmacopoeia 2015 recommends using a complex on-line solid phase extraction method to remove irbesartan before the sample is analyzed by IC columns.[6]

† To whom correspondence should be addressed.
E-mail: yanzhengyujiang@sina.com

High performance liquid chromatography (HPLC) is by far the most common chromatographic technique in use for the analysis of a given sample, because of advantages that include ease of use with gradient elution, compatibility with various samples, and versatility of the retention mechanism, thus allowing the rapid establishment of suitable experimental conditions.[3,7-10] However, conventional reverse phase liquid chromatography (RPLC) columns are not recommended for the determination of highly polar compounds, because such analytes are not sufficiently retained to be well separated because of their high hydrophilicity. The use of precolumn derivatization can enhance the retention of some polar compounds such as 3-aminopiperidine on the RPLC column.[11] Nevertheless, derivatization procedures are usually complex and time consuming. Cation exchange chromatography (CEC) is an alternative to RPLC for the separation of highly polar basic compounds.[3,4,12-15] CEC provides longer retention times and unique selectivity compared to RPLC for highly polar basic compounds. CEC is a good choice for the determination of strong UV-absorbing compounds. CEC, however, offers limited scope for detecting strong polar basic compounds without UV chromophores, since the buffer salts used with CEC for strong cation exchange, such as phosphates, are incompatible with universal detectors. Hydrophilic interaction liquid chromatography (HILIC) has been long reported[16] to be useful for the analysis of polar compounds, including sugars, natural products, and drugs.[17-22] However, the solubility of polar

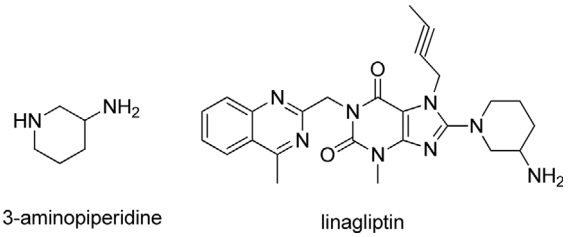

Fig. 1 Chemical structures of 3-aminopiperidine and linagliptin.

compounds may be a limitation in high concentrations of the organic solvent, which is usually used as the initial mobile phase in HILIC. Mixed-mode column is another choice for the analysis of highly polar basic compounds.[23-26] Such columns are usually used with volatile buffer solutions; thus, they can be used with universal detectors for the analysis of polar basic compounds that lack UV-absorbing chromophores.

3-Aminopiperidine is a highly polar basic compound and proves difficult for detection by a UV detector. It is an important starting material in the synthesis of linagliptin (Fig. 1), which has been approved as a DPP-4 inhibitor for the treatment of type II diabetes.[27] The determination of 3-aminopiperidine in the drug sample is of critical importance for quality control. Few methods have been reported for the analysis of such a sample. Babu *et al.*[11] used a chiral HPLC-UV method, involving a precolumn derivatization technique with *para*-toluene sulfonyl chloride (PTSC), to estimate the enantiomeric impurity of 3-aminopiperidine. However, the sample required derivatization before analysis. Another method was proposed by Meek *et al.*, using HPLC coupled with a refractive index detector to directly assay the 3-aminopiperidine dihydrochloride and its enantiopurity.[28] In the current study, a method involving a mixed-mode column combined with a charged aerosol detector (CAD) was developed for the analysis of 3-aminopiperidine in linagliptin. Chromatographic conditions, including the separation mode, type of columns, and mobile phase composition, were optimized. Moreover, different types of detectors, CAD and UV detector, were compared. Finally, the optimized method was used for the determination of 3-aminopiperidine in linagliptin.

Experimental

Apparatus and reagents

The chromatographic system contained an LPG-3400SD pump, WPS-3000 TSL autosampler, TCC-3000RS column oven, and Veo RS CAD. Data were collected and analyzed by Chromeleon Ver. 7.2. All the mentioned instruments and the workstation were from Thermo Fisher (Sunnyvale, CA, USA). The Acclaim Trinity P1 column (150 × 3.0 mm, 3 µm), Acclaim C18 column (150 × 4.6 mm, 5 µm), and Acclaim XAmide (150 × 4.6 mm, 5 µm) were also from Thermo Fisher (Sunnyvale, CA, USA). The Chiralpak AD-H (250 × 4.6 mm, 5 µm) column was from Daicel (Tokyo, Japan).

HPLC-grade acetonitrile, methanol, and ethanol were purchased from TEDIA (Ohio, USA). Ammonium formate (99.995%) was obtained from Acros (NJ, USA). Ammonium acetate (98%) and diethylamine (analytical grade) were purchased from Sigma (St. Louis, MO, USA). Ultra-pure water was produced in-house (Millipore, Advantage A10, USA). Linagliptin was provided by JiangSu Deyuan Pharmaceutical Co., Ltd. (JiangSu, China). 3-Aminopiperidine dihydrochloride was purchased from J&K Scientific (98%, China). Nitrogen (99.999%) was purchased from Shengtang (Tianjin, China). All the other chemicals and reagents used were of analytical grade, unless indicated otherwise.

Preparation of standard stock solution and working solutions

The standard stock solution of 3-aminopiperidine was prepared by weighing 17.29 mg of the 3-aminopiperidine dihydrochloride in a 10 mL volumetric flask. Then 1 mL was transferred to an individual 20 mL volumetric flask and diluted to volume with water, giving a concentration of 50 µg mL^{-1} of 3-aminopiperidine. The standard stock solution was stored at room temperature and the required concentrations of working solutions were then prepared by serial dilution using standard stock solution with water.

Preparation of sample solution

An appropriate amount of linagliptin was transferred separately into individual 10 mL volumetric flasks, diluted to volume with methanol, giving a final concentration of 5 mg mL^{-1}. The sample solution was stored at room temperature.

Chromatographic conditions

Chromatography was performed using an Acclaim Trinity P1 column. Solvent A was acetonitrile, solvent B was 30 mM ammonium formate, and solvent C was water. For the analysis of linagliptin, mobile phase A (ACN) increased from 10 to 50% while mobile phase C (water) decreased from 60 to 20% and mobile phase B held constant at 30% for 16 min. The flow rate was 0.7 mL min^{-1}. The injection volume was 20 µL and the column oven temperature was 30°C. The CAD was set at an evaporation temperature of 55°C and a gas pressure of 62 psi. The acquisition frequency was 10 Hz. The mobile phases used for Acclaim C18 and Acclaim XAmide were methanol:water (5:95, v/v) and acetonitrile:water (95:5, v/v), respectively, and the other conditions were the same as for the Acclaim Trinity P1 column.

Chromatographic conditions for precolumn derivatization method

The precolumn derivatization analysis was performed on a Chiralpak AD-H. The mobile phase consisted of 0.1% diethyl amine in ethanol. The detection wavelength was set at 228 nm. The column temperature was maintained at 27°C. The injection volume was 5 µL. The flow rate was 0.5 mL min^{-1}.

Results and Discussion

Effects of detector and column type

UV detectors are the most widely used detectors in the pharmaceutical industry, because they present a wide range of linearity, good repeatability, and high sensitivity.[7,13,29] For the detection of 3-aminopiperidine, however, UV detectors provide a poor response, because 3-aminopiperidine has no chromophores (see Fig. S1, Supporting Information). The CAD is reported to be a universal detector and provides a good response towards non-volatile compounds.[30-32] Therefore, the CAD was used for optimizing the chromatographic conditions for 3-aminopiperidine in the experiments described in the following sections.

3-Aminopiperidine was first analyzed on a conventional ODS column, Acclaim C18, using methanol:water (5:95, v/v) as the mobile phase (Fig. 2A). The retention for the compound in this case was insufficient for good separation, because of its high

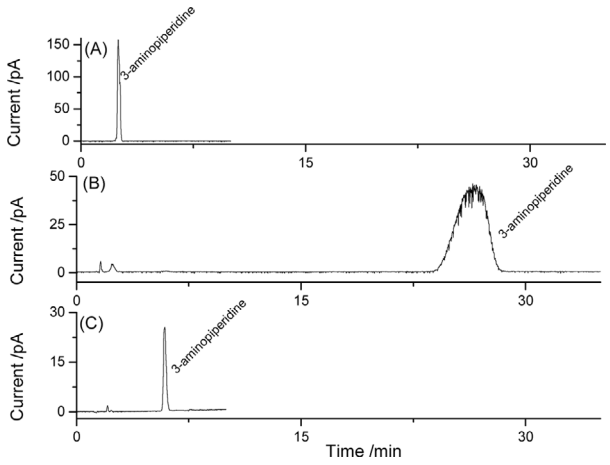

Fig. 2 Analysis of 3-aminopiperidine with Acclaim C18 (A), Acclaim XAmide (B), and Trinity P1 (C). The mobile phase composition is described in the chromatographic conditions section.

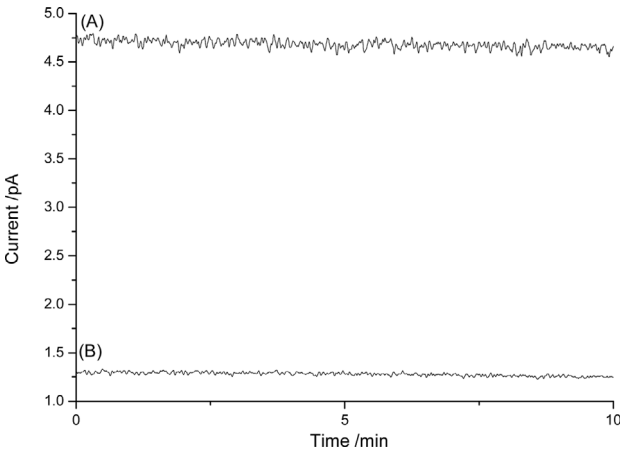

Fig. 3 Background current of the CAD obtained with the HILIC column (A) and Trinity P1 (B). The mobile phase was 30% acetonitrile dissolved in water (isocratic elution).

hydrophilicity. Trifluoroacetic acid (TFA) or formic acid (FA) is widely used as a volatile additive and can have ion-pairing capability in reversed-phase chromatographic conditions. Then, mobile phases containing 0.05% TFA and 0.1% FA were used on Acclaim C18 columns, respectively, but there was still no sufficient retention for 3-aminopiperidine to be well separated (see Fig. S2, Supporting Information). Ion-pairing reagents such as hexane sulfonate can enhance the retention of polar basic compounds.[33] However, such reagents usually modify the columns and lead to poor repeatability of the columns. Furthermore, they are incompatible with universal detectors such as the CAD. Hence, an ion-pairing reagent was not used in this study. HILIC is an alternative to RPLC for the separation of polar compounds.[34] We tested the separation of 3-aminopiperidine on an HILIC column, Acclaim XAmide. A much longer retention was observed on this column (Fig. 2B) than on the C18 column. Apart from HILIC columns, mixed-mode columns such as Trinity P1 have been reported for the analysis of polar compounds.[23] As shown in Fig. 2C, sufficient retention of the target compounds can also be obtained on Trinity P1.

Although both HILIC and mixed-mode analyses can be used to achieve sufficient retention for 3-aminopiperidine, the peak areas and signal-to-noise ratios obtained with these two methods are very different. In the HILIC mode, a high percentage of organic solvent is used, to afford better transport efficiency of the nebulizer. Consequently, a greater number of particles reach the chamber and a larger peak area is obtained, as compared to the case when the mobile phase has low concentrations of organic solvents.[30,35,36] However, bleeding of the HILIC column is much higher than that for the other columns (Fig. 3A), and the CAD is sensitive to column bleed.[37] In other words, high column bleed will lead to high background, large baseline noise, and a low signal-to-noise ratio. Compared to the case of the HILIC column, a mixed-mode column shows much lower bleed (Fig. 3B). A lower concentration of organic solvent was used in the mixed-mode column than in the HILIC mode, leading to low response for 3-aminopiperidine; however, a higher signal-to-noise ratio was obtained in the mixed-mode (S/N = 154) method than that in the HILIC mode (S/N = 130), because of a lower baseline noise of the mixed-mode column. Therefore, the mixed-mode column Trinity P1 was selected for 3-aminopiperidine analysis.

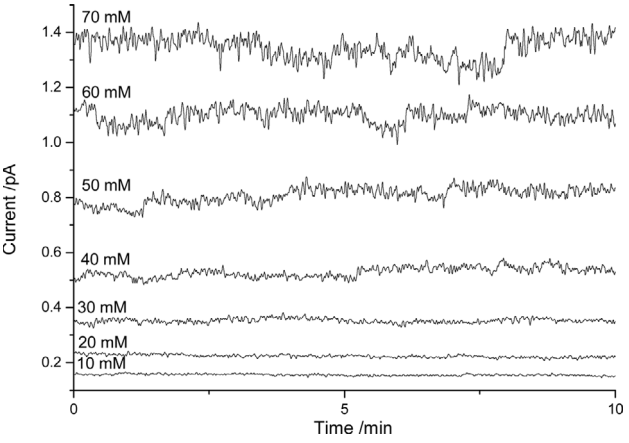

Fig. 4 Background current of the CAD obtained with various concentrations of ammonium formate.

Optimization of chromatographic conditions for 3-aminopiperidine and linagliptin

Linagliptin is soluble in methanol (approximately 60 mg/mL) and sparingly soluble in ethanol (approximately 10 mg/mL).[38] So, methanol was preferred as the solvent. Volatile salts such as ammonium formate and ammonium acetate can be used for the CAD. Long *et al.* reported that a better signal-to-noise ratio can be obtained with ammonium formate than with ammonium acetate.[31] Thus, ammonium formate was used in this study, and its concentration was optimized. Lower concentrations of the buffer solution lead to a smaller background current in the CAD and afforded high sensitivity (Fig. 4). Thus, 10 mM ammonium formate was used as a mobile phase B to separate the target compounds (Fig. 5A). However, 3-aminopiperidine and linagliptin were co-eluted, rendering 10 mmol ammonium formate unsuitable for the determination of 3-aminopiperidine in linagliptin samples. In the mixed-mode, three kinds of interactions, namely hydrophobic, cation exchange, and anion exchange interactions, contributing to the retention of ionic compounds.[4,33] Thus, the retention of ionic compounds could be manipulated by changing the concentration of the organic solvent or buffer solution. It can be seen in Fig. 5B that good

resolution is acquired using 30 mM ammonium formate as a mobile phase B. Good resolution of the target compounds can also be achieved by increasing the initial concentration of the organic solvent. However, the use of acetonitrile was minimized as it was toxic and expensive, and 30 mM ammonium formate was used for the determination of 3-aminopiperidine in linagliptin samples. A higher concentration of buffer solution was not investigated, since the increase in the buffer solution concentration leads to an increase in baseline noise.

Validation of the methods

The quantitative aspects of the proposed methods involving the precision, linearity, limit of detection (LOD), limit of quantification (LOQ), and recovery were examined.

The CAD system generates a parabolic calibration curve and when the concentration is very low (5 – 250 μg mL^{-1}) or it has a narrow range (4 orders of magnitude), the calibration curve is close to being linear for many routine analytical studies.[39,40] Seven concentrations of the 3-aminopiperidine sample, ranging from 0.675 to 173 μg mL^{-1} (*i.e.*, 0.675, 2.73, 5.41, 21.63, 43.25, 86.5, and 173 μg mL^{-1}), were used to obtain calibration curves in this study. The injection volume was 20 μL. The peak areas for three injections of each of the seven standard solutions were plotted *versus* the concentration of 3-aminopiperidine. Standardization curves of the resulting data were then fitted to a linear trend. The dynamic range of the detector was demonstrated to be 2.73 to 86.5 μg mL^{-1}, with a correlation coefficient (R^2) of 0.999. Wide linearity was observed in the current method with CAD. The LOD and LOQ of the current method were 1.35 μg mL^{-1} (S/N = 6.7) and 2.73 μg mL^{-1} (S/N = 16.7), respectively. The results indicate that this method is capable of detecting 1.35 μg mL^{-1} level of 3-aminopiperidine in the drug substance, which is equivalent to about 27 μg of the 3-aminopiperidine per gram of API (270 ppm). The LOD and LOQ of the derivatization method were 0.1 μg mL^{-1} (S/N = 3.2, 200 ppm) and 0.3 μg mL^{-1} (S/N = 10.5, 600 ppm), respectively. The sensitivity of the proposed method meets the requirement for the residue detection of 3-aminopiperidine (Table 1).

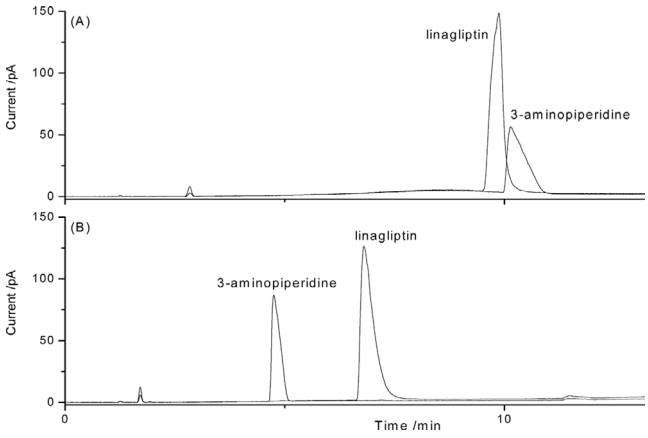

Fig. 5 Analysis of 3-aminopiperidine and linagliptin with 10 mM ammonium formate (A) and 30 mM ammonium formate (B) as solvent B. Other solvents and gradient conditions have been stated in the chromatographic conditions section.

The recovery of 3-aminopiperidine was evaluated with three concentrations, 4.3 (close to the concentration of LOQ),[34] and 51 μg mL^{-1}. As shown in Table 1, the recoveries obtained with these three concentrations are high and overall average recoveries range from 94 to 102%. The recovery of the derivatization method was also investigated (Table 1). Compared to the derivatization method, the current method shows better recovery with various concentrations.

An experiment conducted over two days with six points was performed with a standard solution of 5 μg mL^{-1} to test repeatability. The raw peak areas were analyzed for a total of 18 unique injections over the two-day study. The inter-day and intra-day results for the peak areas were 0.73 and 1.05%, respectively. The precision of the derivatization method was also investigated (Table 1). Compared to the derivatization method, the current method proved to offer better precision.

The robustness of the method was investigated by deliberately changing the chromatographic conditions. The most important parameter to be studied was the amount of 3-aminopiperidine detected in linagliptin. The flow rate of the mobile phase was changed from 0.7 to 0.6 mL min^{-1}, and 0.8 mL min^{-1}. Additionally, the column temperature was changed from 30 to 25°C and 35°C. Finally, the evaporation temperature of the CAD was set from 55 to 50°C and 60°C. There was no significant difference in the results obtained for any of these variations, indicating good robustness of the proposed LC method (Table 2).

Table 1 Comparison of results obtained with the proposed method and precolumn derivatization method for 3-aminopiperidine in the linagliptin sample using the charged aerosol and UV detectors

Item	Proposed method	Precolumn derivatization method
Detector	CAD	UV
Column	Acclaim Trinity P1 column (3.0 × 150 mm, 3 μm)	Chiralpak AD-H (250 × 4.6 mm, 5 μm)
Sample preparation	Directly	Precolumn derivatization
Sample concentration	5 mg mL^{-1} (methanol)	0.5 mg mL^{-1} (1% diethylamine methanol)
Analysis time	16 min	25 min
LOQ	2.73 μg mL^{-1} (equivalent to 0.05% sample concentration)	0.3 μg mL^{-1} (equivalent to 0.06% sample concentration)
LOD	1.35 μg mL^{-1} (equivalent to 0.02% sample concentration)	0.1 μg mL^{-1} (equivalent to 0.02% sample concentration)
Linearity	2.73 – 86.5 μg mL^{-1}	0.2 – 3.03 μg mL^{-1}[c]
Recovery (mean ± SD)	94.19 ± 0.49 (50% identification limit),[b] 99.59 ± 1.25 (100% identification limit), 101.0 ± 1.56 (150% identification limit)	93.72 ± 2.44 (50% identification limit), 95.23 ± 1.38 (100% identification limit), 92.61± 1.32 (150% identification limit)
Precision[a]	0.73% (inter-day), 1.05% (intra-day)	1.34% (inter-day), 2.28% (intra-day)

a. By RSD of the peak areas (n = 6). b. Threshold of impurity for the structure to be identified within the new drug substance according to the ICH Q3a guideline. c. As reported in reference for 3-aminopiperidine.[11]

Table 2 Robustness evaluation of the proposed method

Factor	Chromatographic parameter	Added/ μg mL^{-1}	Found/ μg mL^{-1}	Recovery, %[a]
Optimal	Normal	4.998	4.954	99.12
Low level	Flow rate (mL/min)	4.998	4.871	97.46
	Oven column (°C)	4.998	5.014	100.3
	Evaporation temperature (°C)	4.998	4.936	98.76
High level	Flow rate (mL/min)	4.998	4.924	98.52
	Oven column (°C)	4.998	5.003	100.1
	Evaporation temperature (°C)	4.998	4.822	96.48

a. Recovery of 3-aminopiperidine [%] = amount found/ amount added.

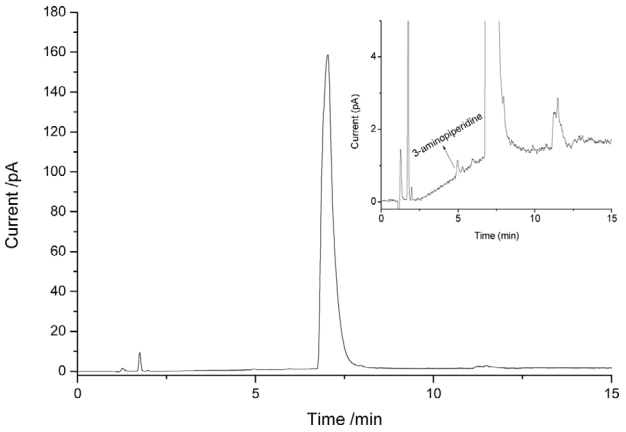

Fig. 6 Analysis of 3-aminopiperidine in linagliptin by using the proposed method.

Determination of 3-aminopiperidine in linagliptin and comparison with precolumn derivatization method

Linagliptin is a DPP-4 inhibitor. It has been used for the treatment of type II diabetes, since it was approved by the U.S. Food and Drug Administration on 3 May 2011.[27] 3-Aminopiperidine is the starting material used in the synthesis of linagliptin and is one of the residual compounds in the linagliptin product. Thus, the determination of 3-aminopiperidine is of critical importance for the quality control of linagliptin. In this study, a linagliptin bulk sample was analyzed with the new proposed method. Three replicate experiments were carried out under the same conditions, and each sample was analyzed by HPLC in triplicate. Using linear calibration of the proposed method, the linagliptin sample was found to contain 0.9 mg g^{-1} of 3-aminopeperidine (Fig. 6). The linagliptin sample was found to contain 0.7 mg g^{-1} of 3-aminopeperidine using the precolumn derivatization method. The recovery of the precolumn derivatization using the HPLC-UV method was lower than that using the proposed HPLC-CAD method and, therefore, the analytical results for the linagliptin sample using the proposed method were slightly higher and closer to the true value.

Conclusions

A method utilizing a mixed-mode column combined with a CAD was developed in this study for the determination of 3-aminopiperidine in linagliptin. Chromatographic conditions including the detector, separation mode, and mobile phase composition were optimized. Compared to the precolumn derivatization UV method, the proposed method allowed for more convenient operation, less expense on the column, much shorter analysis time, and a significant decrease in cost and waste generation. Furthermore, a wide range in linearity (2.73 - 86.5 μg mL^{-1}), high sensitivity (LOQ <2.73 μg mL^{-1}), and good precision (RSD of peak area <2%) were offered by the method. In addition, the parameters such as buffer solution and column bleed that affected the sensitivity of the CAD were investigated. This is a new non-derivative, highly sensitive, and robust method for the determination of 3-aminopiperidine, constituting a new application of the CAD for the quantitative analysis of highly polar basic compounds.

Acknowledgements

We thank Jiangsu Deyuan Pharmaceutical Co., Ltd for their technical assistance. We would like to acknowledge Thermo Fisher Scientific Co., Ltd. in China for research support.

Supporting Information

This material is also available online at http://www.jsac.or.jp/analsci/.

References

1. A. Chattopadhyay, R. Rukmini, and S. Mukherjee, *Biophys. J.*, **1996**, *71*, 1952.
2. Z.-W. Lai, C. Li, J. Liu, L. Kong, X. Wen, and H. Sun, *Eur. J. Med. Chem.*, **2014**, *83*, 547.
3. Z. Long, Z. Guo, X. Xue, X. Zhang, and X. Liang, *J. Sep. Sci.*, **2013**, *36*, 3845.
4. Z. Long, Z. Guo, X. Xue, X. Zhang, L. Nordahl, and X. Liang, *Anal. Chim. Acta*, **2013**, *804*, 304.
5. D. C. Johnson, D. Dobberpuhl, R. Richard, and P. Vandeberg, *J. Chromatogr. A*, **1993**, *640*, 79.
6. Chinese Pharmacopoeia Commission, "Pharmacopoeia of the People's Republic of China", **2015**, Vol. 1, Medical Science Press, Beijing, China, 397.
7. Y. Liu, X. Xue, Z. Guo, Q. Xu, F. Zhang, and X. Liang, *J. Chromatogr. A*, **2008**, *1208*, 133.
8. Z. Guo, C. Wang, T. Liang, and X. Liang, *J. Chromatogr. A*, **2010**, *1217*, 4555.
9. J. Wei, A. Shen, H. Wan, J. Yan, B. Yang, Z. Guo, F. Zhang, and X. Liang, *J. Sep. Sci.*, **2014**, *37*, 1781.
10. K. Li, W. Zhu, Q. Fu, Y. Ke, Y. Jin, and X. Liang, *Analyst*, **2013**, *138*, 3313.
11. C. V. R. Babu, N. R. Vuyyuru, K. P. Reddy, M. V. Suryanarayana, and K. Mukkanti, *Chirality*, **2014**, *26*, 775.
12. Z. Long, C. Wang, Z. Guo, X. Zhang, L. Nordahl, and X. Liang, *J. Chromatogr. A*, **2012**, *1256*, 67.
13. Z. Long, C. Wang, Z. Guo, X. Zhang, L. Nordahl, J. Zeng, J. Zeng, and X. Liang, *Analyst*, **2012**, *137*, 1451.
14. Z. Long, Y. Zhang, Z. Guo, L. Wang, X. Xue, X. Zhang, S. Wang, Z. Wang, O. Civelli, and X. Liang, *Planta Med.*, **2014**, *80*, 1124.
15. Z. Long, D. Yu, Y. Liu, N. Du, Y. Tao, L. Mei, Z. Guo, and X. Liang, *Anal. Chim. Acta*, **2015**, *872*, 77.
16. A. J. Alpert, *J. Chromatogr. A*, **1990**, *499*, 177.
17. V. V. Tolstikov and O. Fiehn, *Anal. Biochem.*, **2002**, *301*, 298.

18. Y. Guo and S. Gaiki, *J. Chromatogr. A*, **2005**, *1074*, 71.
19. P. Hemstrom and K. Irgum, *J. Sep. Sci.*, **2006**, *29*, 1784.
20. M. Godejohann, *J. Chromatogr. A*, **2007**, *1156*, 87.
21. D. V. McCalley, *J. Chromatogr. A*, **2007**, *1171*, 46.
22. C. Apostolou, C. Kousoulos, Y. Dotsikas, and Y. L. Loukas, *Biomed. Chromatogr*., **2008**, *22*, 1393.
23. X. Liu and C. A. Pohl, *J. Chromatogr. A*, **2012**, *1232*, 190.
24. F. Al-Rimawi and U. Pyell, *J. Chromatogr. A*, **2007**, *1160*, 326.
25. E. Apfelthaler, W. Bicker, M. Laemmerhofer, M. Sulyok, R. Krska, W. Lindner, and R. Schuhmacher, *J. Chromatogr. A*, **2008**, *1191*, 171.
26. X. Dong, A. Shen, Z. Gou, D. Chen, and X. Liang, *Carbohyd. Res.*, **2012**, *361*, 195.
27. M. Eckhardt, E. Langkopf, M. Mark, M.Tadayyon, L. Thomas, H. Nar, W. Pfrengle, B. Guth, R. Lotz, P. Sieger, H. Fuchs, and F. Himmelsbach, *J. Med. Chem.*, **2007**, *50*, 6450.
28. G. A. Meek, S. K. I. P. Kunhimon, R. Shankar, V. H. Dahanukar, T. K. Moha, M. B. Wagh, A. K. Pal, V. M. B. Meesala, and S. Shrivastava, U. S. Patent, **2013**, 20130172562 A1.
29. C. Wang, Z. Guo, Z. Long, X. Zhang, and X. Liang, *J. Chromatogr. A*, **2013**, *1281*, 60.
30. C. Brunelli, T. Gorecki, Y. Zhao, and P. Sandra, *Anal. Chem.*, **2007**, *79*, 2472.
31. Z. Long, Z. Guo, I. Acworth, X. Liu, Y. Jin, X. Liu, L. Liu, and L. Liang, *Talanta*, **2016**, *151*, 239.
32. Z. Long, Z. Guo, X. Liu, Q. Zhang, X. Liu, Y. Jin, L. Liang, H. Li, J. Wei, and N. Wu, *Talanta*, **2016**, *146*, 423.
33. H. Luo, L. Ma, C. Paek, and P. W. Carr, *J. Chromatogr. A*, **2008**, *1202*, 8.
34. A. Shen, Z. Guo, X. Cai, X. Xue, and X. Liang, *J. Chromatogr. A*, **2012**, *1228*, 175.
35. C. Crafts, B. Bailey, M. Plante, and I. Acworth, *J. Chromatogr. Sci.*, **2009**, *47*, 534.
36. M. Ligor, S. Studzinska, A. Horna, and B. Buszewski, *Crit. Rev. Anal. Chem.*, **2013**, *43*, 64.
37. T. Teutenberg, J. Tuerk, M. Holzhauser, and T. K. Kiffmeyer, *J. Chromatogr. A*, **2006**, *1119*, 197.
38. R. Agrawal, P. Jain, and S. N. Dikshit, *Curr. Drug Targets.*, **2012**, *13*, 970.
39. T. Vehovec and A. Obreza, *J. Chromatogr. A*, **2010**, *1217*, 1549.
40. S. Matsuyama, Y. Orihara, S. Kinugasa, and H. Ohtani, *Anal. Sci.*, **2015**, *31*, 61.