Journal of Chromatography B, 1033 (2016) 112–116

Contents lists available at ScienceDirect

Journal of Chromatography B

journal homepage: www.elsevier.com/locate/chromb

Short communication

Self-initiated and concentration-dependent degradation of tetracaine in neat standard solutions: A trouble-shooting story

Aimin Tan*, Yanxin Wu, Guifen Gu, John C. Fanaras

Nucro-Technics, Scarborough, ON, Canada

ARTICLE INFO

Article history:
Received 5 July 2016
Received in revised form 3 August 2016
Accepted 12 August 2016
Available online 12 August 2016

Keywords:
Tetracaine
Stability
Stock solution
LC–MS
Bioanalysis

ABSTRACT

This paper presents the trouble-shooting for a very unusual stability case. Tetracaine was found unstable in neat solutions only at high concentrations, but not at low concentrations. Moreover, its stable-isotope labeled internal standard did not show similar behavior. A series of trouble-shooting experiments were conducted to uncover the root cause. Some generally applicable precautions/insights can be drawn from this investigation to avoid potential stability issues during bioanalytical method development and validation.

© 2016 Elsevier B.V. All rights reserved.

1. Introduction

Tetracaine (Fig. 1), a local anesthetic drug, is commonly used to reversibly block nerve function. The instability of tetracaine in biological matrices (e.g. plasma) due to esterase activities is well-known. Enzyme inhibitors, like neostigmine bromide and physostigmine salicylate, are commonly used to inhibit the esterase activities [1–3]. However, no stability issues were ever reported for tetracaine in neat standard solutions (stock and working standard solutions) for bioanalytical methods [1–3].

Since neat standard solution stability is also an important requirement in regulatory guidance for bioanalytical method validation [4,5], the stability of tetracaine in stock and working standard solutions was evaluated during method development to ensure a good chance of successful method validation. Surprisingly, a stability issue was observed for the stock solution of tetracaine (500 μg/ml) when it was kept at ambient room temperature, but not for its stable-isotope labeled internal standard (IS), tetracaine-d_6, which is supposed to have the same or very similar physico-chemical properties as the analyte [6]. Moreover, a diluted tetracaine standard solution (5 μg/ml, 100-fold dilution of the stock solution with the stock solution solvent) did not show the stability issue under the same storage conditions. This was also unexpected because a stability issue would normally affect either both high and low concentrations or mainly the low concentration, but not the other way around.

Intrigued by the aforementioned observations, we conducted a series of trouble-shooting experiments to find the root cause, which resulted in very interesting findings. It is hoped that these findings and the insights drawn from this investigation can be used to avoid potential stability issues during bioanalytical method development and validation for many similar situations.

2. Experimental

2.1. Chemicals and reagents

Tetracaine and its IS, tetracaine-d_6 chloride, were purchased from Sigma-Aldrich (Oakville, Ontario Canada) and C/D/N Isotopes (Pointe-Claire, Quebec, Canada), respectively. Acetonitrile (HPLC grade), methanol (HPLC grade), and ammonium acetate (ACS grade) were obtained from Caledon Laboratories (Georgetown, Ontario, Canada). Formic acid (ACS grade) and perfluoropentanoic acid (NFPA, 97%) were bought from Sigma-Aldrich. USP type water was produced in-house using a Milli-Q Plus Ultra-Pure Water System (Millipore Canada, Etobicoke, Ontario, Canada).

2.2. Preparation of analyte and internal standard solutions

Several stock solutions of tetracaine at the concentration of 500 μg/ml were prepared in methanol/H_2O (50/50, v/v), methanol,

* Corresponding author.
E-mail addresses: tan@nucro-technics.com, dr.aimintan@gmail.com (A. Tan).

http://dx.doi.org/10.1016/j.jchromb.2016.08.019
1570-0232/© 2016 Elsevier B.V. All rights reserved.

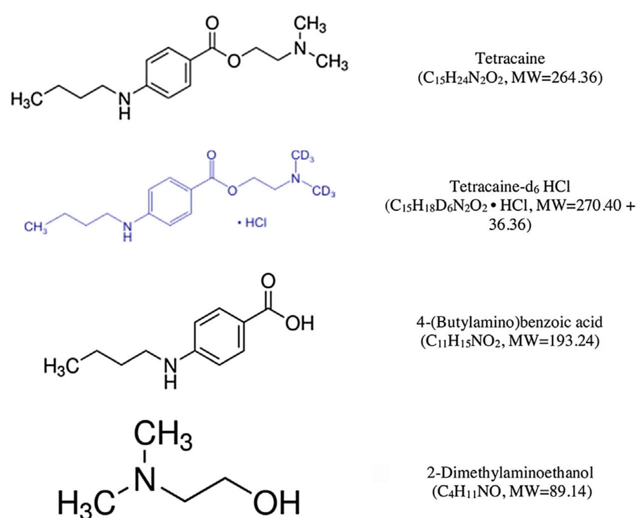

Fig. 1. Chemical structures and molecular weights (MW) of tetracaine, tetracaine-d_6 hydrochloride, 4-(butylamino)benzoic acid, and 2-Dimethylaminoethanol (MW: Molecular weight).

and acetonitrile. The stock solution of the IS was prepared in methanol/H_2O (50/50, v/v) at the concentration of 150 μg/ml.

2.3. Sample preparation

No complicated sample extraction procedure (e.g. liquid-liquid extraction) was required because only neat stock or working standard solutions were involved in this investigation. Various neat solution samples were simply diluted. For stability evaluations, aliquots of stock solutions or working standard solutions were first stored at various testing conditions as per Table 1. Then, both comparison and stability aliquots were diluted in the same way in the mobile phase to the same nominal concentrations (e.g. 100 ng/ml) for injection on an LC–MS/MS system. For identification of degradation products, stability samples were diluted only 10-fold in the mobile phase to augment the chance for detecting potential degradation products.

2.4. LC–MS/MS analysis

An Agilent 1200 series liquid chromatograph coupled with an Agilent 6410 Triple Quad LC/MS (Agilent Technologies Canada, Mississauga, Ontario, Canada) were used for the LC–MS/MS analyses. A 10 μl aliquot of diluted standard solution samples was injected onto an ACE Excel 5 Super C18 column (3.0 × 75 mm, 5 μm, Canadian Life Science, Peterborough, ON, Canada) maintained at 25 °C for isocratic separation (flow rate at 0.5 ml/min). The mobile phase was acetonitrile/H_2O/formic acid/NFPA, 45/54.8/0.1/0.1 (v/v/v/v) containing 5 mM ammonium acetate. Tetracaine and its IS were eluted at 2.5 min. The run time for the stability evaluations was 3.5 min whereas it was extended to 5 or 10 min for the characterization of potential degradation products.

For stability evaluation, the MS detection was in the positive electrospray ionization mode using the precursor to product ion transitions of m/z 265.2 → 176.1 and 271.2 → 176.1 with dwell times of 300 ms and 200 ms for tetracaine and the IS, respectively. The fragmentor and collision energy were set at 78 V and 14 V, respectively. The source parameters (gas temperature, gas flow, nebulizer, and capillary) were set at 350 °C, 13 l/min, 60 psi, and 4000 V, respectively. For characterization of potential degradation products, the parent ion scans were performed in the positive electrospray ionization mode from 80 to 300 amu. The product ion scans were conducted from 50 to 300 amu in the positive ionization mode.

2.5. Data acquisition, chromatogram integration, and quantification

Data acquisition and quantification were performed using Agilent MassHunter software (Versions B.04.01 and B.04.00, respectively).

3. Results and discussion

3.1. How did the stability issue start?

Based on the literature [3] and an existing method from another laboratory, the stock solutions of tetracaine and tetracaine-d_6 were first prepared in methanol/H_2O (50/50, v/v). Both compounds solubilized well in this solvent. When the short-term stability of the two compounds at ambient room temperature was initially evaluated, there was no stability issue for stock and working solutions of the stable-isotope labelled IS. Additionally, no stability issue was observed for the low-concentration analyte working solution at 5 μg/ml (Table 1). However, the analyte stock solution (500 μg/ml) showed a relatively large % of difference (−8.3%). Although this would still meet the acceptance criteria (±10%) that are adopted for the evaluation of solution stabilities, the difference of −8.3% contrasted sharply with those near 0% differences for the IS and low-concentration tetracaine standard solutions.

Since the difference in solution stability between an analyte and its stable-isotope labelled IS as well as the difference between low and high concentrations were unexpected, the test was repeated to rule out any experimental errors. For a slightly longer stability duration (28 h vs. 24 h in the initial test), the % of difference reached −12.2% (Table 1), which confirmed the stability issue of tetracaine stock solution. Hence, an investigation was deemed necessary to figure out what caused the difference in neat solution stability between tetracaine and its stable-isotope labeled IS and between high and low concentrations.

3.2. Stability of tetracaine in different solvents

To check if there was any difference among different stock solution solvents, tetracaine stock solutions were subsequently prepared in acetonitrile and methanol. Then, similar stability tests were performed for both high and low concentrations. No significant degradation was observed for acetonitrile- or methanol-based stock and working standard solutions (Table 1). Therefore, the degradation must be associated with the existence of water. In other words, the most likely cause for the degradation was hydrolysis.

3.3. Impact of acidification on stability of tetracaine

Since tetracaine is a base, it was hypothesized that the pH of neat tetracaine standard solutions in methanol/H_2O (50/50, v/v) would be concentration-specific. Indeed, when the pH of 500 μg/ml and 5 μg/ml standard solutions were measured, a significant difference was observed. The pH of tetracaine stock solution (500 μg/ml) was 8.6, whereas that of the diluted tetracaine standard solution (5 μg/ml) was 7.8. This difference in pH may explain the difference in solution stability of tetracaine between high and low concentrations.

Based on this, it was anticipated that the acidification of the stock solution would reduce the degradation of tetracaine. When the 50% methanol-based stock solution was acidified with formic

Download English Version:

https://daneshyari.com/en/article/1211872

Download Persian Version:

https://daneshyari.com/article/1211872

Daneshyari.com