Journal of Pharmaceutical and Biomedical Analysis 56 (2011) 633–636

Contents lists available at ScienceDirect

Journal of Pharmaceutical and Biomedical Analysis

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

Short communication

Boron phenylalanine and related impurities: HPLC analysis, stability profile and degradation pathways

Lindsay Dick[a], Neil Dooley[a], Moira A. Elliott[a], Steven J. Ford[a,*], Malcolm R. Gordon[b], Gavin W. Halbert[a], William J. Kerr[b]

[a] Strathclyde Institute for Pharmacy and Biomedical Analysis, Robertson Building, University of Strathclyde, 161 Cathedral Street, Glasgow G4 0RE, United Kingdom
[b] Department of Pure & Applied Chemistry, Thomas Graham Building, 295 Cathedral Street, Glasgow G1 1XL, United Kingdom

ARTICLE INFO

Article history:
Received 24 March 2011
Received in revised form 23 June 2011
Accepted 27 June 2011
Available online 1 July 2011

Keywords:
BPA
BNCT
HPLC
Impurities
Degradation

ABSTRACT

Boron phenylalanine is one of the lead drug candidates in the field of Boron Neutron Capture Therapy. Its inherent low toxicity allows large doses to be administered, but this makes it important to identify, rationalise and quantify impurities. Here we report a chromatographic assay method, the conditions under which the parent compound is unstable, and the suggested degradation mechanisms.

1. Introduction

Boron neutron capture therapy (BNCT) is a two stage cancer treatment [1] comprising of initial tumour loading with a boron-10 containing compound followed by localised neutron beam irradiation. The resulting boron neutron capture leads to spontaneous α-particle emission, destroying the immediate surrounding tumour. Therapeutically the concentration of boron-10 needs to be as high as possible, so large doses of loading agent are required to 'saturate' the tumour. Cancer Research UK is currently clinically investigating the use of boron phenylalanine, BPA (Fig. 1) for the treatment of glioma. The authors conducted analytical method development and stability testing for the BPA, but because of the large doses the characterisation of impurities becomes a key issue. Other chromatographic methods for BPA have been published [2,3], but in addition to our method we also report the outcomes of forced degradation and long terms stability studies, as well as suggested mechanistic pathways. The authors believe this information is important for establishing BPA synthesis and purification schemes, storing BPA long term and 'solution handling' during quantification assays. Both tyrosine and phenylalanine have been reported as impurities by previous workers [4], and while both have low toxicities, the large BPA doses (potentially >50 g) mean impurity 'control' and 'quantification' are pertinent clinical issues.

2. Materials and methods

2.1. Chemicals

Mobile phase components were HPLC grade or better and were purchased commercially. Degradation reagents were laboratory grade or better and were purchased commercially. Water for injection (WFI) was supplied by Baxters (Newbury, UK). BPA and its synthetic intermediates – (2-(4-Bromophenyl)-[1,3]dioxane (BrPD); 4-Formylbenzeneboronic acid (FBBA); 2-tert-Butoxycarbonylamino-3-[4-(5,5-dimethyl-[1,3,2]dioxaborinan-2-yl)-phenyl]acrylic acid methyl ester (BDPA) – were supplied by Hammercap Medical AB (Sweden).

2.2. HPLC method

HPLC assays were performed on Surveyor HPLC systems (Thermo, Hemel Hempstead, UK). Mobile phase A was trifluoroacetic acid:water:methanol (0.1:85:15, v/v/v), mobile phase B was methanol. The mobile phase gradient was 100% A for 9 min, linear increase to 100% B at 27 min, followed by 17 min equilibration at 100% mobile phase A. Flow rate was 1.0 ml/min over a Luna C18 15 cm × 4.6 mm id (Phenonenex, Maccelesfield, UK) held at 25 °C. Detection wavelengths were 230, 256 and 270 nm. Nominal work-

* Corresponding author. Tel.: +44 141 548 2454; fax: +44 141 548 4903.
E-mail address: steven.ford@strath.ac.uk (S.J. Ford).

0731-7085/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.jpba.2011.06.025

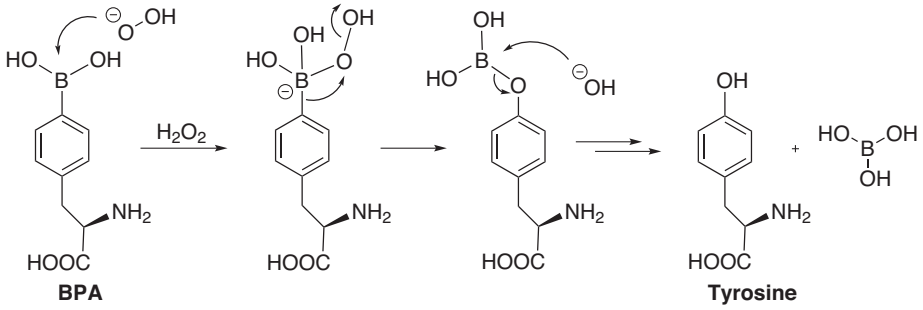

Fig. 1. Mechanistic pathway showing the oxidative degradation of BPA to tyrosine.

ing concentration (NWC) for the BPA, tyrosine and phenylalanine was set to 0.5 mg/ml, 5 μg/ml and 5 μg/ml respectively, with final samples prepared in 100 mM acetic acid. In the case of drug product, mannitol had no effect on BPA peak area response. Response factors (RF), where quoted, are calculated by linear gradients and expressed in units of Pa ml/ng. An example trace is shown in Fig. 2.

2.3. Sample preparation and degradation assays

BPA drug product was generated by freeze drying BPA (100 mg/ml) with mannitol (110 mg/ml), pH adjusted to 8.0 ± 0.1 in WFI, with sample vials being incubated in the dark at 4, 25 and 40 °C for several months. BPA raw powder was incubated in the dark at 25 °C, 40 °C and 55 °C, without further processing. BPA forced degradation tests were performed using BPA dissolved in 100 mM NaOH, 100 mM HCl or 5% $FeCl_3$ and these samples were incubated at 55 °C for 24 h. A BPA solution in 6 mM H_2O_2 sample was prepared immediately prior to HPLC analysis.

3. Results and discussion

3.1. Separation

BPA and tyrosine are quantified at 230 nm with retention times of 5.3 and 4.5 min respectively. Phenylalanine elutes at 11.0 min and is quantified at 256 nm. (See Fig. 2.) BrPD and FBBA are detected at 256 nm and elute at 17.3 and 23.7 min respectively. BDPA is detected at 270 nm, but co-elutes with FBBA. The BPA peak gives peak purity values of >0.997 under forced degradation and control conditions.

3.2. Quantification

BPA linearity and repeatability at both wavelengths was shown to have $R^2 > 0.99$, with %RSD < 0.55% over 80–120% of the BPA NWC: the RF's at 230 nm and 256 nm were 33.4 and 17.2 respectively. The tyrosine linearity and repeatability at 230 nm was shown to have $R^2 > 0.99$, with %RSD < 0.1% over 25–200% of the tyrosine NWC and a RF of 28.8. The phenylalanine linearity and repeatability at 256 nm was shown to have $R^2 > 0.98$, with %RSD < 12% over 25–200% of the phenylalanine NWC and a RF of 0.883. All three synthetic impurities were detectable at concentrations of 0.5 μg/ml (or 0.1% of the BPA NWC) both in the presence and absence of BPA, and neither BrPD, or a FBBA/BDPA combination were observed in the available BPA samples.

3.3. Degradation

As a raw powder, BPA is stable, producing no detectable degradation when stored at 55 °C for 6 months, or 40 °C for 12 months. BPA was also observed to be stable in acidic and $FeCl_3$ solutions (the latter testing metal-catalysed degradation). BPA degradation to tyrosine was observed under alkali and oxidative conditions and in the latter case this occurred extremely rapidly (mass balance was observed). BPA/mannitol lyophilised drug product showed a slow, temperature dependent degradation to phenylalanine, generating

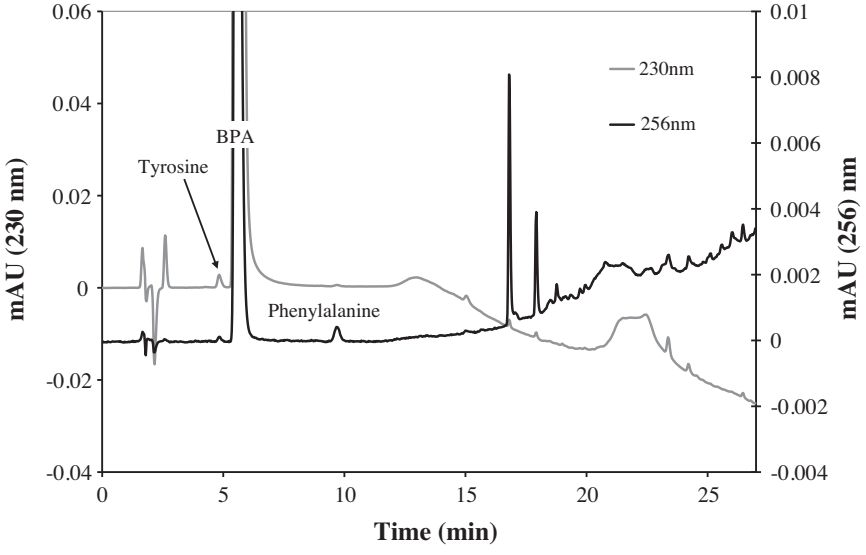

Fig. 2. A typical chromatographic trace of BPA, showing low levels of tyrosine and phenylalanine impurities.

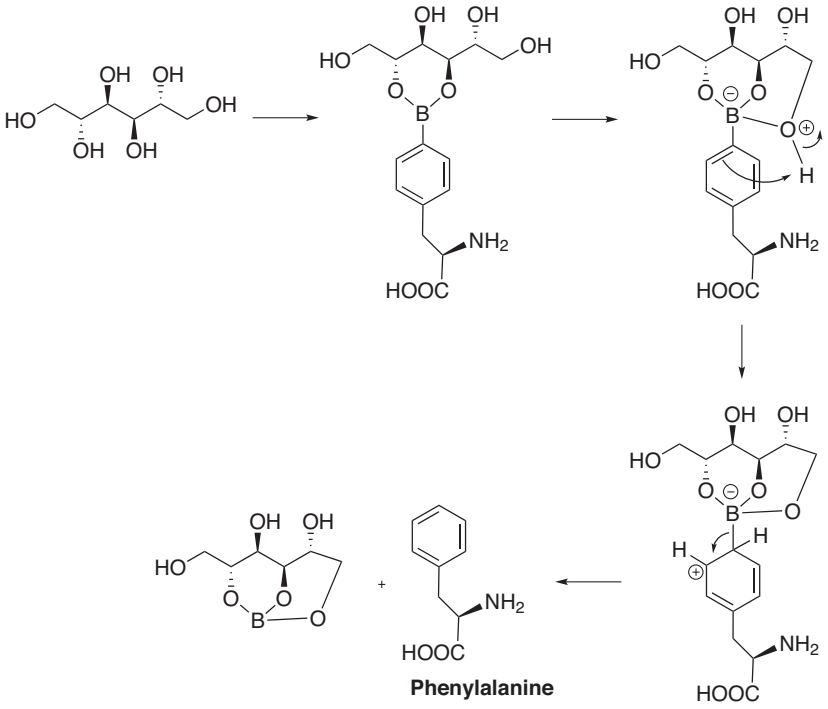

Fig. 3. Mechanistic pathway showing the mannitol-mediated degradation of BPA to phenylalanine.

approximately 1% of phenylalanine (with respect to BPA mass) at 40 °C over 6 months.

3.4. Degradation mechanisms

3.4.1. Acidic degradation

Aryl ring systems, especially electron-rich boronic acids, are reported to undergo protodeborylation under aqueous acidic conditions [5,6]. However, the observed levels of BPA stability shown here may be due to the thermal and acidic conditions employed in this study being appreciably milder than those used previously.

3.4.2. Oxidative degradation

Oxidative degradation of boronic acids in aqueous hydrogen peroxide is a well known and quantified process, widely accepted to proceed through the mechanistic pathway delineated in the scheme shown in Fig. 1 [5,7,8]. An initial co-ordination of the peroxide (either as the neutral species, followed by proton loss, or as the anionic form), initiates the donation of a lone pair into the empty p-orbital of the electron-deficient boron, forming the borate species. Subsequent aryl migration promotes the loss of hydroxide, which can then co-ordinate back into the neutral boron species and facilitates the release of tyrosine and boric acid.

3.4.3. Alkaline degradation

The observed degradation of BPA to tyrosine in aqueous alkaline solutions is a process which has some previous literature precedent for aryl boronic acids [9,10]. The actual process involved in this transformation and the mechanistic detail has as yet not been elucidated, though previous reported examples indicate that the transformation is accelerated by the exposure of the alkaline solution to air, suggesting that an oxidative process is responsible. Having stated all of this, protodeborylation of the aryl boronic acid is by far the most common outcome under aqueous alkaline conditions, i.e. the formation of the parent arene [5,11].

3.4.4. Solid state mannitol degradation

The observation that lyophilised BPA/mannitol samples partially degraded to phenylalanine when stored for long periods of time is considered consistent with literature precedent (relating to storage in fructose [3]). It is suspected that the observed protodeborylation is occurring in a stepwise process (similar to that envisaged as part of acid-promoted hydrolysis). More generally, boronic acids are known to form boronate esters as an equilibrium process; as water is removed from the system, the equilibrium is pushed towards the condensed boronic ester [9]. In this case the equivalent boronic mannitol ester is expected to form (see the mechanistic scheme shown in Fig. 3). Previous studies by Kulvik et al. [4] and Shull et al. [12] have shown than under dehydrating conditions BPA readily forms thermodynamically favoured borate esters with fructose. Since mannitol is a polyol (or reduced sugar) in its open-chain alcohol form, the free hydroxyl sites could feasibly form a similar borate arrangement [13–15]. The subsequent borate is then suspected of undergoing a rate-limiting hydrogen transfer and fast elimination to gradually form phenylalanine [16–18].

4. Conclusions

With large doses of BPA being administered to cancer patients, both accurate quantification and 'chemical control' of impurity levels (even of low toxicity impurities such as tyrosine and phenylalanine) are important objectives. The chromatographic method, storage conditions and mechanistic schemes presented here play crucial roles in achieving those goals.

In this study we have shown that while the reductive stability of BPA is consistent with early reports [5], the alkali, oxidative and acidic degradation mechanisms are more unexpected. This study demonstrates that workers using alkaline solutions to assist BPA dissolution should only do so under mild and controlled conditions.

The degradation of BPA/mannitol lyophilised samples is reported and discussed. Importantly, for other researchers, the long term stability of the raw BPA powder is also reported.

Acknowledgements

Cancer Research UK for funding N.D., M.A.E., S.J.F. and G.W.H. Dr. Nigel Westwood (Cancer Research UK, Drug Development Office, London) and Syntagon AB (Sweden) for material supplies. M.R.G. and W.J.K. thank the Engineering and Physical Sciences Research Council (EPSRC) and GlaxoSmithKline for research support. Formulation Unit technical team for practical assistance.

References

[1] R.F. Barth, J.A. Coderre, M. Graca, H. Vicente, T.E. Blue, Boron neutron capture therapy of cancer: current status and future prospects, Clin. Cancer Res. 11 (2005) 3987–4002.
[2] J. Vahatalol, J. Tuominen, J. Kokkonen, O. Kriz, S. Karonen, M. Kallio, Trace impurities identified by high performance liquid chromatography/electrospray mass spectrometry in two different synthetic batches of 4-boronophenylalanine, Rapid Commun. Mass Spectrom. 12 (1998) 1118–1122.
[3] E.A. Gautier, M.J. Roberti, R.T. Gettar, R.J. Rebagliati, D.A. Batistoni, Assessment of chemical purity of 10B-enriched p-boronophenylalanine by high-performance liquid chromatography coupled on-line with inductively coupled plasma optical emission spectrometry, Anal. Bioanal. Chem. 388 (2007) 499–503.
[4] M. Kulvik, J. Vahatalo, E. Buchar, M. Farkkila, E. Jarviluoma, J. Jaaskelainen, O. Kriz, J. Laakso, M. Rasilainen, I. Ruokonen, M. Kallio, Clinical implementation of 4-dihydroxyborylphenylalanine synthesised by an asymmetric pathway, Eur. J. Pharm. Sci. 18 (2003) 155–163.
[5] M.F. Lappert, Organic compounds of boron, Chem. Rev. (1956) 959–1064.
[6] A.H. Soloway, Stability and synthesis of phenyl boronic acid, J. Am. Chem. Soc. 81 (1959) 3017–3019.
[7] H.G. Kuivila, Electrophilic displacement reactions. III. Kinetics of the reaction between hydrogen peroxide and benzeneboronic acid, J. Am. Chem. Soc. 76 (1954) 870–874.
[8] H.C. Brown, C. Snyder, B.C.S. Rao, G. Zweifel, Organoboranes for synthesis. 2. Oxidation of organoboranes with alkaline hydrogen peroxide as a convenient route for the cis-hydration of alkenes via hydroboration, Tetrahedron 42 (1986) 5505–5510.
[9] D.G. Hall, Boronic Acids, Preparations and Applications in Organic Synthesis and Medicine, first ed., Wiley-VCH, Wienhiem, 2005.
[10] S. Soundararajan, E.N. Deusler, J.H. Hageman, Structure of 4-carboxy-2-nitrobenzeneboronic acid, Acta Crystallogr. C: Cryst. Struct. Commun. (1993) 690–693.
[11] A.D. Ainley, F. Challenger, Studies of the boron-carbon linkage. Part I. The oxidation and nitration of phenyl-boric acid, J. Chem. Soc. (1930) 2171–2180.
[12] B.K. Shull, D.E. Spielvogel, G. Head, R. Gopalaswamy, S. Sankar, K. Devito, Studies on the structure of the complex of the boron neutron capture therapy drug, L-p-boronophenylalanine, with fructose and related carbohydrates: chemical 13C NMR evidence for the β-D-fuctofuranose 2,3,6-(p-phenylalanylorthoboronate) structure, J. Pharm. Sci. 89 (2000) 215–222.
[13] N. Geffen, R. Semiat, M.S. Eisen, Y. Balazs, I. Katz, C.G. Dosoretz, Boron removal from water by complexation to polyol compounds, J. Membr. Sci. 286 (2006) 45–51.
[14] S. Mothana, J.-M. Grassot, D.G. Hall, Multistep phase-switch synthesis by using liquid-liquid partitioning of boronic acids: productive tags with an expanded repertoire of compatible reactions, Angew. Chem. Int. Ed. 49 (2010) 2883–2887.
[15] H.G. Kuivila, A.H. Keough, E.J. Soboczenski, Areneboronates from diols and polyols, J. Org. Chem. 19 (1954) 780–783.
[16] H.G. Kuivala, K.V. Nahabedian, Electrophilic displacement reactions. X. General acid catalysis in the protodeboronation of areneboronic acids, J. Am. Chem. Soc. 83 (1961) 2159–2163.
[17] H.G. Kuivala, K.V. Nahabedian, Electrophilic displacement reactions. XI. Solvent isotope effects in the protodeboronation of areneboronic acids, J. Am. Chem. Soc. 83 (1961) 2164–2166.
[18] K.V. Nahabedian, H.G. Kuivala, Electrophilic displacement reactions. XII. Substituent effects in the protodeboronation of areneboronic acids, J. Am. Chem. Soc. 83 (1961) 2167–2174.