



ELSEVIER

Contents lists available at ScienceDirect

MethodsX

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

Method Article

Validation of a stability-indicating HPLC-UV method for the quantification of acetazolamide in Oral-Mix and Oral-Mix SF



Charlotte Gillium, Mihaela Friciu, Nicolas Abatzoglou, Grégoire Leclair*

Platform of Biopharmacy, Faculty of Pharmacy, Université de Montréal, Montréal, Canada

A B S T R A C T

This manuscript details the modifications made to the HPLC assay method described in the USP monograph for Acetazolamide Compounded Oral Suspension.

- The method was modified to allow the quantification of acetazolamide in two new suspension vehicles: Oral Mix and Oral Mix SF;
- It was validated for linearity, accuracy, precision and specificity;
- It was demonstrated stability-indicating and suitable for use in a stability study using these vehicles.

© 2020 The Authors. Published by Elsevier B.V.

This is an open access article under the CC BY license. (<http://creativecommons.org/licenses/by/4.0/>)

A R T I C L E I N F O

Method name: Stability-indicating HPLC-UV method for acetazolamide in Oral-Mix and Oral-Mix SF.*Keywords:* HPLC-UV, Acetazolamide, Oral suspension, Stability, Compounding, Extemporaneous preparation*Article history:* Received 30 October 2019; Accepted 20 February 2020; Available online 27 February 2020

Specification Table

Subject Area:	Pharmacology, Toxicology and Pharmaceutical Science
More specific subject area:	Oral suspensions, compounding, extemporaneous preparations, stability studies
Method name:	Stability-indicating HPLC-UV method for acetazolamide in Oral-Mix and Oral-Mix SF
Name and reference of original method:	United States Pharmacopeia 42 – National Formulary 37: Acetazolamide Oral Suspension. Rockville (MD): United States Pharmacopeial Convention; 2018.
Resource availability:	<i>No other resource is available.</i>

* Corresponding author.

E-mail address: gregoire.leclair@umontreal.ca (G. Leclair).

Method details

Overview

Acetazolamide is a drug indicated for the treatment of edema, epilepsy, mountain sickness and glaucoma [1–3]. It is available in the USA and in Canada in various solid oral dosage forms [4,5]. When a liquid oral dosage form is required, it can be prepared extemporaneously according to the USP monograph for Acetazolamide Compounded Oral Suspension [6]. New liquid oral acetazolamide formulations were developed using Oral Mix and Oral Mix SF suspension vehicles and a stability-indicating method was required in order to conduct a stability study for these new formulations. This manuscript details the modifications made to the USP methods for the quantification of acetazolamide in Oral Mix and Oral Mix SF vehicles. It also details the validation of this new method.

Materials and method

Materials

Acetazolamide bulk powder (lot 602553/B, exp. May 2019), Oral Mix (Lot I185/A, exp. January 2018), Oral Mix SF (Lot H1136, exp. October 2017) were graciously provided by Medisca Pharmaceutique Inc., Montreal, QC, Canada.

Methanol (HPLC grade, lot 144689), acetonitrile (HPLC grade, lot 141693), hydrochloric acid aqueous solution (1 M, lot 160432), sodium hydroxide aqueous solution (1 M, lot 143309), hydrogen peroxide aqueous solution (3%, lot 135515) were purchased from Fisher Scientific, Ottawa, ON, Canada.

Heptafluorobutyric acid (HFBA, lot A2716) was purchased from Santa Cruz Biotechnology, Dallas, TX, USA.

Water was purified using a Milli-Q Synthesis A10 system, Millipore, Etobicoke, ON, Canada.

Mobile phase A consisted of an aqueous solution of HFBA (0.15%) and mobile phase B was an acetonitrile solution of HFBA (0.15%).

All materials were used without further purification.

Instrumentation

The HPLC system (Prominence UFLC, Shimadzu, Laval, QC, Canada) consisted of a SIL-20AC HT refrigerated autosampler, a DGU-20A5 solvent degasser, a LC-20AD binary pump, a CTO-20AC column oven and an SPD-M20A photodiode array detector. The HPLC column was a Zorbax SB-C18 (4.6 × 150 mm, 5 μm, P/N 883975-902, S/N USCM010053, Agilent, Santa Clara, CA, USA).

Sample preparation

Acetazolamide suspensions were prepared from acetazolamide bulk powder using either Oral Mix or Oral Mix SF at a concentration of 25 mg mL⁻¹ in a mortar using a pestle.

Samples for HPLC injections were prepared by diluting the acetazolamide suspension (50 μL) using methanol (450 μL) in a 1.5-mL centrifuge tube. The mixture was vortexed (20 s) and then centrifuged (9400 g, 10 min). Supernatant (20 μL) was further diluted using mobile phase A (480 μL), vortexed (20 s) and transferred to a sealed 96-well plate. These solutions for injection had a nominal concentration of 100 μg mL⁻¹ and were analysed immediately after preparation.

HPLC-UV conditions

The injection volume was 10 μL and all injections were conducted in duplicate. A variation of more than 0.5% between replicates triggered an investigation to identify instrumental malfunction.

The autosampler was refrigerated (5 °C) and the column oven was heated to 40 °C.

A flow gradient was used at a constant flow rate (1.0 mL min⁻¹): 0 min (mobile phase A, 88: mobile phase B, 12), 4 min (88: 12), 4.5 min (70: 30), 5.5 min (70: 30), 6 min (88: 12), 13 min (88: 12).

Table 1
Accuracy.

Nominal conc. ($\mu\text{g mL}^{-1}$)	HPLC response in vehicle	HPLC response in mobile phase	Recovery vehicle:mobilephase (%)	Back calculated conc. ($\mu\text{g mL}^{-1}$)	Back calculated recovery (%)
Acetazolamide in Oral Mix					
25	714288	698669	102.2	24.6	98.4
50	1447021	1398513	103.5	49.8	99.6
75	2141535	2099685	102.0	73.7	98.3
100	2954532	2808259	105.2	101.7	101.7
150	4345057	4214417	103.1	150.0	100.0
Acetazolamide in Oral Mix SF					
25	699705	698669	100.1	24.4	97.6
50	1417084	1398513	101.3	49.5	99.0
75	2137448	2099685	101.8	74.6	99.5
100	2806614	2808259	99.9	98.0	98.0
150	4347873	4214417	103.2	151.8	101.2

Detection wavelength and retention time were 265 nm and 3.3 min, respectively.

Method validation

In order to define the validation parameters of this method, including (1) Linearity and range, (2) accuracy, (3) precision and (4) specificity, several reports and guidelines pertaining to the validation of stability-indicating HPLC methods were considered [7–15].

Limits of detection and quantification were not formally evaluated as the lower concentration of the validation range was clearly above the limit of quantification based on signal to noise ratio. This method is not intended to be used outside of its validated range.

Since robustness was not evaluated, this method requires calibration i. Furthermore, the injection of quality control samples every 20 test samples will confirm the stability of the system throughout injection runs. This stability check was applied when assessing the stability of acetazolamide oral suspensions over a period of 90 days.

Linearity and range

Acetazolamide suspensions (25 mg mL^{-1}) were prepared in Oral Mix and Oral Mix SF as described above. These suspensions ($500 \mu\text{L}$) were diluted using methanol (4.5 mL), vortexed (20 s) and then centrifuged (9400 g , 10 min). Samples (20 , 40 , 60 , 80 and $120 \mu\text{L}$) of the supernatant (2.5 mg mL^{-1}) were further diluted using mobile phase A (1980 , 1960 , 1940 , 1920 , $1880 \mu\text{L}$) and vortexed (20 s) to obtain standard solutions at concentrations of 25 , 50 , 75 , 100 and $150 \mu\text{g/mL}$ prior to injection in triplicate.

HPLC response was plotted as a function of acetazolamide concentration for both Oral Mix and Oral Mix SF. A linear regression was calculated for both plots (y -intercept was forced through zero). Coefficient of determination were respectively 0.9997 for Oral Mix and 0.9995 for Oral Mix SF.

The target concentration of test samples is $100 \mu\text{g mL}^{-1}$. The range of this method is 25 – $150 \mu\text{g mL}^{-1}$, or 25 – 150% of the target concentration.

Accuracy

Standard solutions of acetazolamide in mobile phase A were prepared at concentrations of 25 , 50 , 75 , 100 and $150 \mu\text{g mL}^{-1}$ and analysed using the HPLC-UV method. As listed in Table 1, in order to evaluate the effects of the matrix as well as sample preparation, the areas of the acetazolamide peaks were compared to the areas obtained during the validation of linearity and range described above. Furthermore, the concentration of acetazolamide of these samples was back calculated using the linear regression slope parameter and compared with their nominal value to establish the accuracy of the linear regression at all tested concentrations.

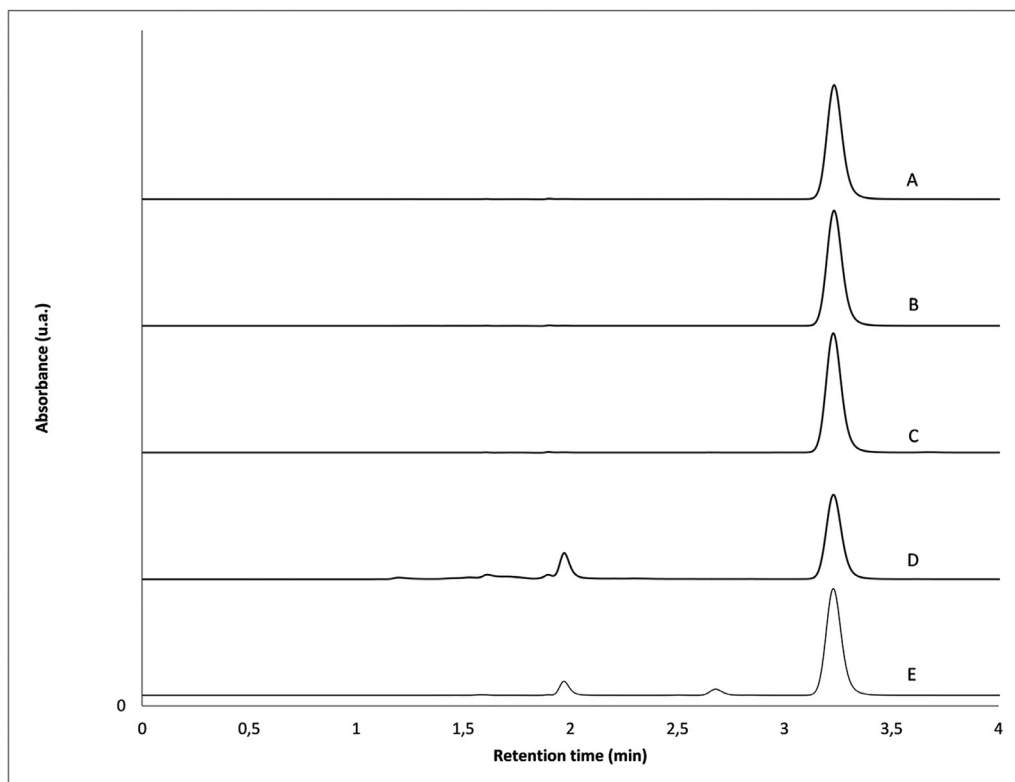


Fig. 1. Representative chromatograms. (A) Acetazolamide standard solution in an aqueous solution of HFBA 0.15% (mobile phase A); (B) acetazolamide suspension prepared from bulk drug using Oral Mix vehicle; (C) acetazolamide suspension prepared from bulk drug using Oral Mix SF; acetazolamide suspension in mobile phase A after either alkaline (D, NaOH 0.1%, 60 °C, 3 h) or acidic (E, HCl 0.1%, 60 °C, 3 h) degradation. Degradation in water and peroxide did not yield any additional peak (data not shown).

The effect of sample preparation and matrix on the accuracy of the method resulted in recoveries ranging from 99.9% to 105.2% at all tested concentrations over the investigated range. Furthermore, the back calculated recovery varied from 97.6% to 101.7% at all tested concentrations over the method range.

Precision

Intraday precision corresponds to the coefficient of variation of the triplicated analyses performed for the validation of linearity and range detailed above. Interday variability is similarly calculated from analyses performed on three different days. These results are reported in [Table 2](#).

The precision of the method evaluated as the intraday CV and the interday CV were not more than 0.17% and 4.75%, respectively, at any of the tested concentrations over the method range.

Specificity

An acetazolamide suspension (25 mg mL^{-1}) was prepared in Oral Mix as described above. Aliquots of this suspension (0.5 mL) were mixed with water (0.5 mL), aqueous hydrogen peroxide 3% (0.5 mL), aqueous hydrochloric acid 1 M (0.5 mL) and aqueous sodium hydroxide 1 M (0.5 mL). These four solutions were stored for 3 h at 60 °C. The acidic solution (100 μL) was neutralized using aqueous

Table 2
Precision.

Nominal conc. ($\mu\text{g mL}^{-1}$)	Intraday CV (%)	Interday CV (%)
Acetazolamide in Oral Mix		
25	0.17%	1.34%
50	0.14%	2.31%
75	0.07%	2.03%
100	0.05%	1.46%
150	0.09%	1.92%
Acetazolamide in Oral Mix SF		
25	0.05%	4.75%
50	0.03%	3.23%
75	0.05%	2.85%
100	0.02%	3.76%
150	0.12%	3.39%

sodium hydroxide 1 M (50 μL) and diluted using methanol (350 μL). Similarly, the alkaline solution was neutralized using aqueous hydrochloric acid 1 M (50 μL) and diluted using methanol (350 μL). The water and peroxide solutions were directly diluted using methanol (400 μL). All these solutions were vortexed (20 s) and centrifuged (9400 g, 10 min). Supernatants (20 μL) were recovered and diluted in mobile phase A (480 μL) to achieve a nominal concentration of 100 $\mu\text{g mL}^{-1}$ (prior to degradation) and analysed using the HPLC-UV method. The chromatograms obtained from these analyses were compared to the chromatograms obtained from acetazolamide suspensions in Oral Mix and Oral Mix SF (25 mg/mL) submitted to sample preparation for HPLC injection. They were also compared to the chromatograms obtained from a solution of acetazolamide in mobile phase A (100 $\mu\text{g/mL}$).

Recovery values of 102%, 103%, 73% and 92% were observed following degradation in water, peroxide, alkaline and acidic conditions, respectively. As shown in Fig. 1, no peak overlap of acetazolamide with excipients, impurities or degradation products was observed. Acetazolamide peak purity index calculated between 235 and 295 nm was not less than 0.9999 in all cases.

Conclusion

A stability-indicating method for the quantification of acetazolamide in Oral Mix and Oral Mix SF suspensions was developed and validated. This method is intended to be used for the evaluation of the stability of acetazolamide in these two oral suspension vehicles. This methods improves the USP assay method for acetazolamide compounded oral suspension. The USP method was developed and validated for these two vehicles: 1:1 mixture of Vehicle for Oral Solution, NF (regular or sugar-free), and Vehicle for Oral Suspension, NF, or Cherry Syrup, NF. The method reported in this manuscript was specifically developed and validated for acetazolamide compounded oral formulation in two other slightly different commercial vehicles: Oral Mix and Oral Mix SF. On the one hand, the latter method used a lower injection volume (10 vs. 20 μL) and a gradient elution system wich allowed sharper peaks with a better resolution. On the other hand, the USP method provided stronger signals and a simpler isocratic elution system.

Acknowledgments

This research was supported by Medisca Pharmaceutique Inc., Montréal, Quebec, Canada.

Declaration of Competing Interest

This study was funded by an unrestricted grant from Medisca Pharmaceutique Inc., Montréal, Quebec, Canada. The company was not involved in the design or conduct of the study; the collection, management, or interpretation of the data; or the preparation, review, or approval of the manuscript.

References

- [1] W.M. Grant, R.R. Trotter, Diamox (acetazolamide) in treatment of glaucoma, *AMA Arch. Ophthalmol.* 51 (6) (1954) 735–739.
- [2] S.R. Resor, L.D. Resor, Chronic acetazolamide monotherapy in the treatment of juvenile myoclonic epilepsy, *Neurology* 40 (11) (1990) 1677–1681.
- [3] S.A. Forwand, M. Landowne, J.N. Follansbee, J.E. Hansen, Effect of acetazolamide on acute mountain sickness, *N. Engl. J. Med.* 279 (16) (1968) 839–845.
- [4] Drugs@FDA: FDA Approved Drug Products, Database entries for Acetazolamide, Silver Spring, MD, 2019 US Food and Drug Administration <https://www.accessdata.fda.gov/scripts/cder/daf/index.cfm> (Accessed date: 28 October 2019).
- [5] Drug Product Database (DPD), Database Entries for Acetazolamide, Government of Canada, Health Canada, Ottawa, Canada, 2019 <http://www.hc-sc.gc.ca/dhp-mpps/prodpharma/databasdon/index-eng.php> (Accessed date: 28 October 2019).
- [6] United States Pharmacopeia 42–National Formulary 37: Acetazolamide Oral Suspension, United States Pharmacopeial Convention, RockvilleMD, 2018.
- [7] L.A. Trissel, K.P. Flora, Stability studies: five years later, *Am. J. Hosp. Pharm.* 45 (7) (1988) 1569–1571.
- [8] Q. Li, Z. Liu, S. Kolli, et al., Stability of extemporaneous erlotinib, lapatinib, and imatinib oral suspensions, *Am. J. Heal Syst. Pharm.* 73 (17) (2016) 1331–1337.
- [9] M.H. Ensom, D. Décarie, Dexamethasone 1mg/mL suspension prepared from crushed tablets: stability in glass and plastic bottles and plastic syringes, *Can. J. Hosp. Pharm.* 69 (1) (2016) 49–51.
- [10] M. Nahata, Long-term stability of zonisamide, amitriptyline, and glycopyrrolate in extemporaneously prepared liquid-dosage forms at two temperatures, *Int. J. Pharm. Compd.* 20 (2) (2016) 164–166.
- [11] A.O. Ferreira, H.C. Polonini, S.L. Silva, et al., Feasibility of amlodipine besylate, chloroquine phosphate, dapsone, phenytoin, pyridoxine hydrochloride, sulfadiazine, sulfasalazine, tetracycline hydrochloride, trimethoprim and zonisamide in Syrspend® of PH4 oral suspensions, *J. Pharm. Biomed. Anal.* 118 (2016) 105–112.
- [12] S. Vrignaud, T. Briot, A. Launay, et al., Design and stability study of a paediatric oral solution of methotrexate 2mg/ml, *Int. J. Pharm.* 487 (1–2) (2015) 270–273.
- [13] D.L. Prohotsky, K.M. Juba, F. Zhao, Formulation and stability of an extemporaneously compounded oral solution of chlorpromazine HCl, *J. Pain Palliat. Care Pharmacother.* 28 (4) (2014) 367–370.
- [14] B.J. Stanisz, S.K. Paszun, A. Zalewska, Stability of cilazapril in pediatric oral suspensions prepared from commercially available tablet dosage forms, *Acta Pol. Pharm.* 71 (4) (2014) 661–666.
- [15] B. Kommanaboyina, C.T. Rhodes, Trends in stability testing, with emphasis on stability during distribution and storage, *Drug Dev. Ind. Pharm.* 25 (7) (1999) 857–868.