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Spectrophotometric Determination of Losartan Potassium in Tablets

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ABSTRACT

In the quality control of pharmaceutical products, it is of utmost importance that validated analytical methods are used to ensure the credibility of the results generated. At the time of the study, official monographs from the United States Pharmacopeia and National Formulary (USP-NF) for the quantification of Losartan potassium in tablets were unavailable, denoting the need for a validated analytical procedure for the analysis of the drug. The study adapted direct and first-derivative UV spectrophotometry methods proposed by Bonfilio and others (2010) for the assay of Losartan potassium in Losartan 50 mg. capsules, then modified and validated the said procedures for the assay of Losartan potassium in Losartan 100 mg. tablets following the International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) guidelines on method validation for accuracy, precision, specificity, linearity, limit of detection, and limit of quantitation. Results demonstrated that all the performance characteristics of both methods were highly satisfactory and confirmed the possible application of the methods in routine analysis of Losartan potassium tablets.

Keywords: Losartan Potassium, UV spectrophotometric determination, assay validation, direct and first-derivative spectra, hypertension

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SPECTROPHOTOMETRIC DETERMINATION OF LOSARTAN POTASSIUM IN TABLETS

Hypertension is a condition characterized by persistently elevated arterial blood pressure (Wells and others 2009). It is considered as one of the most significant risk factors in the development of heart disease (Wells and others 2009), which has been identified as the leading cause of mortality in the Philippines (World Health Organization 2010).

Angiotensin II receptor blockers (ARB) are among the primary agents used as first line treatment for hypertension with compelling indications. ARBs potently and selectively inhibit angiotensin II generally by competitive binding to the AT_1-receptor. Losartan, an example of ARB, is approved for stroke prophylaxis and is well tolerated in patients with heart failure (Jackson 2006). It is available in two dosage strengths (50 mg.- and 100 mg.-tablet), in different tablet preparations (core and film-coated), and in combination with hydrochlorothiazide (Wai Fun and others 2008).

Although available literature has described several analytical methods for the assay of Losartan at the time of the study, no pharmacopeia has yet described a monograph for Losartan drug products. The absence of a universal procedure deprives the public of an assurance on safety, quality and efficacy; thus, validated analytical procedures are needed to quantify Losartan potassium found in various drug preparations. Two methods are presented in this paper by the researchers for the analysis of Losartan drug products.

This study aimed to validate the conditions and modify, if necessary, the direct and first-derivative UV spectrophotometry methods to quantify Losartan potassium in 100 mg. tablets with an appropriate level of confidence. This study is of significance to the pharmaceutical industry for the routine analysis of Losartan potassium in tablet dosage form. The study also contributes to safeguarding the public against the presence of low-quality Losartan drug products in the market. Lastly, the study provides new and additional knowledge which can be used for future method development for the quantification of Losartan in tablet dosage forms.

The paper of Bonfilio and others (2010) described an analytical procedure for the quantitative analysis of Losartan in the capsule but not in the tablet form. By determining the validity of their analytical procedure as it applies to the quantitative analysis of Losartan in tablets, and applying the International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) validation parameters, the robustness of the methodology developed by Bonfilio and others (2010) were further tested.

The study evaluated the following performance characteristics of the two methods: specificity, accuracy, precision, linearity, range, limit of detection (LOD), and limit of quantitation (LOQ). Due to limitations in resources, the study only used the available spectrophotometer at the College of Pharmacy, University of the Philippines Manila and a single brand of Losartan potassium 100 mg. tablet (Cozaar®) preparation. The study did not include simultaneous determination of possible degradation products or impurities.

METHODS

Sample

Losartan potassium working standard with a purity of 99.8%, Lot 2008-0111-06, and expiration date of October 2011 was used as reference standard and was generously provided by the Institute of Pharmaceutical Sciences, National Institutes of Health, University of the Philippines Manila. Losartan potassium (Cozaar®) tablets, labeled 100 mg. Losartan potassium, were obtained from a local distributor and were used as samples for the experiment. The tablets were described to contain excipients such as microcrystalline cellulose, lactose hydrous, pregelatinized starch, magnesium stearate, hydroxypropyl cellulose, hypromellose and titanium dioxide (Merck and Co. 2010). Starch, magnesium stearate, microcrystalline cellulose, lactose, hydroxyethycellulose, hydroxymethylpropylcellulose and titanium dioxide used in the preparation of the simulated standard of excipients (SSE) were of analytical grade and were provided by the Department of Industrial Pharmacy, College of Pharmacy, University of the Philippines Manila. Locally manufactured distilled water was used as solvent in both standard and sample solutions.

Instrument

A double-beam Genesys 10S UV – Visible Spectrophotometer unit, with a VISIONlite™ SE software and 1.8 nm bandwidth, was used for the spectrophotometric determination. Sartorius AG analytical balance was used to weigh all reagents. All solutions were placed in a 1 cm. quartz cuvette during measurement.

Procedure

The direct and first-derivative spectrophotometric procedures developed and validated by Bonfilio and others (2010) for the assay of Losartan potassium in Losartan 50 mg. capsules were validated in the study for the assay of Losartan potassium in Losartan 100 mg. tablets. Twenty tablets were accurately weighed and powdered. One hundred milligrams (100 mg.) of Losartan potassium was

weighed and placed on a 100 mL-volumetric flask to which 40 mL of distilled water was added. The solution was sonicated and the flask was filled to volume.

The absorbance of the standard solution (1 mg/mL) was measured from 200-300nm for both zero- and first-order derivative spectrophotometry to obtain the λ_{max}. Aliquots of the standard solution equivalent to 0.1-2 mg. Losartan potassium were transferred into 10 mL volumetric flasks. The analytical curve was produced by plotting drug concentration versus the absorbance λ_{max} obtained, for both zero-order and first-order derivative spectrophotometry. The calibration curve was plotted after measuring the concentration and recording the regression equation (Youssef and Taha 2007).

The method validation was performed following ICH specifications for specificity, accuracy, precision, linearity, range, limit of detection, and limit of quantitation.

Specificity

Specificity was determined by comparing the sample solution to a simulated sample of excipients (SSE). The sample solution (1 mg/mL) was spiked with a SSE concentration of 25 ug/mL. Using the Handbook of Pharmaceutical Excipients as reference (Rowe and others 2006), the percentages of the inactive components of the Losartan tablet were approximated (Appendix 1). The SSE mixture, with an amount equal to the weight of one tablet, was mixed with 100 mg. Losartan standard and dissolved in distilled water. Solutions were subsequently prepared following the procedure of Bonfilio and others (2010) to obtain a final concentration of 5 mg/L and 10 mg/L for the direct and first-derivative spectra, respectively, using distilled water as diluent.

Accuracy

Accuracy was determined by recovery of known amounts of Losartan potassium standard added to the sample solution. Sample solutions of 5.0 mg/L and 10 mg/L for the two spectra were mixed with adequate Losartan standard solutions using serial dilutions to obtain a final concentration of 4.0, 5.0 and 6.0 mg/L for the direct spectrophotometry and of 8.0, 10, and 12 mg/L for the first-derivative spectrophotometry. All measurements were done in triplicates.

Precision

Precision was evaluated in terms of repeatability and intermediate precision. For repeatability, six standard solutions of the same concentration were measured twice

on the same day. For intermediate precision, six standard solutions of the same concentration were measured on a different day by a different analyst.The concentrations used were 5.0 mg/L and 10 mg/L solutions for the direct and first-derivative spectra, respectively.

Linearity and Range

Linearity and range were evaluated by using Losartan sample solutions of 0, 4.0, 5.0, 6.0 and 7.0 mg/L for the direct derivative spectra, and 6.0, 8.0, 10, 12, 14 mg/L for the first-derivative spectra to produce an analytical curve.

Limits of Detection and Limits of Quantitation

The limits of detection (LOD) and quantitation (LOQ) were calculated using Equations (1) and (2):

$$LOD = 3\left(\frac{\mathrm{SD}}{\alpha}\right) \quad (1)$$

$$LOQ = 10\left(\frac{\mathrm{SD}}{\alpha}\right) \quad (2)$$

where SD is the standard deviation of the 10 blank readings (distilled water and mobile phase) and α is the calibration curve slope obtained in the linearity.

Data Analysis

All data were entered and analyzed using Microsoft® Office Excel® 2007.

RESULTS AND DISCUSSION

Validation of the assay procedure developed by Bonfilio and others (2010) started with the determination of the wavelength at which absorbance for the direct and first-derivative spectrophotometry was to be measured. The wavelength of maximum absorbance (λ_{max}) of the zero-order spectrum (Appendix 2) obtained using 5.0 mg/L as the 100% nominal concentration was found to be at 205.4 nm. On the other hand, the first-derivative spectrum, calculated and graphed according to the absorbances measured by the instrument, showed an intense negative peak at 234 nm (Appendix 3). At this wavelength, the absorbances of the Losartan potassium standard solution of 4.0, 8.0, 12, 16, and 20 mg/L showed absorbance values of 0.201, 0.393, 0.564, 0.762, and 0.951, respectively. For the subsequent analyses, 10 mg/L was set at the 100% level of the analytical curve in the first-derivative spectrophotometric method so that the analytical signal corresponds to the direct spectrophotometric method. Based on the results, the wavelengths determined for both methods closely corresponded the findings of Bonfilio and

others (2010) of 205 nm for the direct spectrophotometry and 234 nm for the first-derivative spectrophotometry. Thus, subsequent absorbance for the direct spectrophotometry was done at 205.4 nm while absorbance was set at 229 and 239 nm to obtain the data for the first-derivative spectrophotometry.

Following the ICH guidelines on analytical method validation, the adapted procedure from Bonfilio and others (2010) was validated for the assay of Losartan potassium in Losartan 100-mg tablet preparation. To reiterate, the performance characteristics tested were specificity, accuracy, precision (repeatability and intermediate precision), linearity, range, LOQ, and LOD. Results are shown in Table 1 together with the set acceptance criteria.

Table 1. Summary of performance characteristics of direct and first-derivative spectrophotometry

Parameter	Acceptance Criteria	Direct Spectrophotometry			First-Derivative Spectrophotometry		
		Result		Remark	Result		Remark
Specificity*	No significant difference between spiked and unspiked absorbance curves	No significant difference between spiked and unspiked absorbance curves		Passed	No significant difference between spiked and unspiked absorbance curves		Passed
Accuracy	95-105% recovery per concentration level* (90-110%***)	Conc	% recovery#	Passed	Conc	% recovery	Passed
		80%	99.2		80%	104.3	
		100%	98.8		100%	98.5	
		120%	96.9		120%	101.1	
Precision**	RSD<5%			Passed			Passed
	Overall repeatab.	2.21%			3.28%		
	Intermed. precision	3.17%			3.77%		
Linearity	$R^2 \geq 0.99$****	1.00		Passed	1.00		Passed
	P-value of y-intercept >0.05*****	0.66		Passed	0.54		Passed
Range*	80-120% nominal concentration	% nominal conc	Conc (mg/L)	Passed	% nominal conc	Conc (mg/L)	Passed
		80	4.00		80	8.00	
		100	5.00		100	10.00	
		120	6.00		120	12.00	
LOD*	Variable	0.03 mg/L		Passed	0.61 mg/L		Passed
LOQ*	Variable	0.10 mg/L		Passed	1.86 mg/L		Passed

* Adapted from ICH 2006
** Adapted from Brazil, as cited in Bonfilio and others 2010
*** Adapted from Brazilian Pharmacopeia
**** Adapted from Bryan 2009
***** Adapted from Chan and others 2004

#- %recovery formula: % = (actual concentration/theoretical concentration) x 100

In both methods, none of the excipients displayed any absorbance upon being spiked to a standard solution of known concentration. The excipients also did not interfere with the measurement of the Losartan potassium contained in the solution as shown in the zero-order spectrum (Appendix 4) and first-order spectrum (Appendix 5) of the unspiked and spiked standard solutions. These findings indicate that absorbance at 205.4 nm for the direct spectrophotometry, and at 229 and 239 nm for the first-derivative spectrophotometry, is specific for Losartan potassium.

Both methods exhibited good accuracy with a mean recovery range of 96.9-104.3 for the direct spectrophotometric method and 98.5-101.1 for the first-derivative method. Being specific and accurate, both direct and first-derivative methods are therefore suitable for the determination of Losartan potassium in tablets.

The results of the precision study conformed to the acceptance criteria, with the overall repeatability value of 2.2120% and 3.2837%, for the direct and first-derivative spectrophotometry, respectively, and intermediate precision of 3.1740% and 3.7659%, respectively, for the two methods. The methods were therefore expected to be insensitive to small changes in conditions (i.e., sample preparation, weighing, dilution, time, operator).

Linearity was evaluated by linear regression. The coefficient of determination (R^2) values obtained from direct spectrophotometric method over the range of 4.0-6.0 mg/L was 1.00 and the equation produced was A=0.0949C-0.0068. For the first-derivative spectrophotometric method, the R^2 value obtained over the range of 8.0-12.0 mg/L was 1.00 and the calibration equation was dA/dW = -0.0018C-0.0002. The method was found to be linear, with the absorbance response being directly proportional to the concentration of Losartan.

Using the formula for LOD and LOQ, the limits of quantitation and detection were calculated to be 0.10 and 0.03 mg/L, respectively, for direct spectrophotometry whereas limits of quantitation and detection for first-derivative spectrophotometry were calculated to be 1.86 and 0.61 mg/L, respectively. The results indicate that the analyses were performed beyond the quantitation limit.

In summary, all performance characteristics were found to be highly satisfactory. These results confirmed the suitability of both methods for the assay of Losartan potassium in Losartan 100 mg. tablet preparation. No modifications to the procedure developed by Bonfilio and others (2010) were necessary to conform to the set acceptance criteria. Robustness and inter-laboratory studies are recommended to provide further evidence for the applicability of both methods for routine analysis of Losartan potassium in various Losartan tablet preparations.

APPENDICES

Appendix 1. Composition of the simulated sample of excipients

Table 2. Simulated Sample of Excipients*

Ingredients	Amount
Starch	0.6000 g
Magnesium stearate	0.1500 g
Microcrystalline cellulose	0.6000 g
Lactose	1.3500 g
Hydroethylcellulose	0.1922 g
Hydroxymethylpropylcellulose	0.0600 g
Titanium dioxide	0.0481 g
TOTAL	3.0003 g

* Composition based on Rowe RC, Sheskey PJ, Quinn ME 2009

Appendix 2. Losartan potassium zero-order absorption spectrum

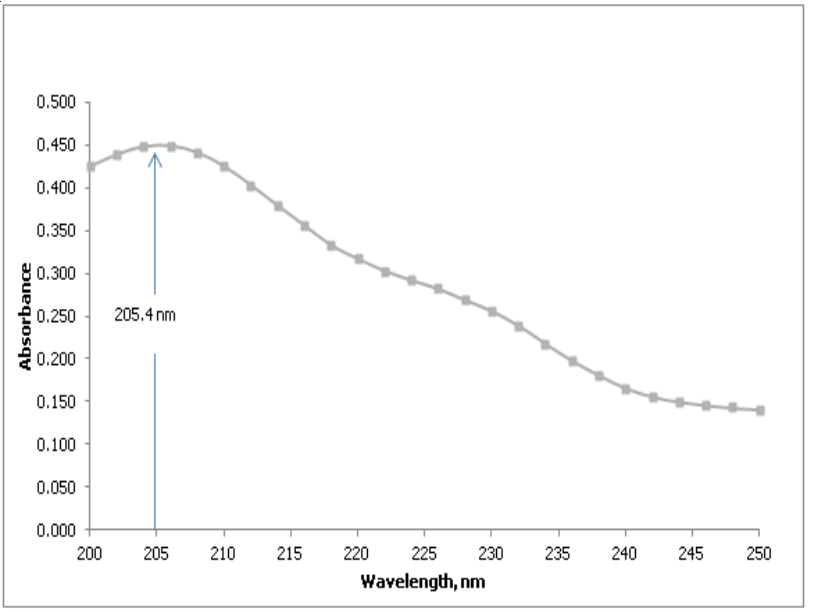

Figure 1. Losartan potassium zero-order absorption spectrum at 5 mg/L using distilled water as solvent.

Appendix 3. Losartan potassium first-order absorption spectrum

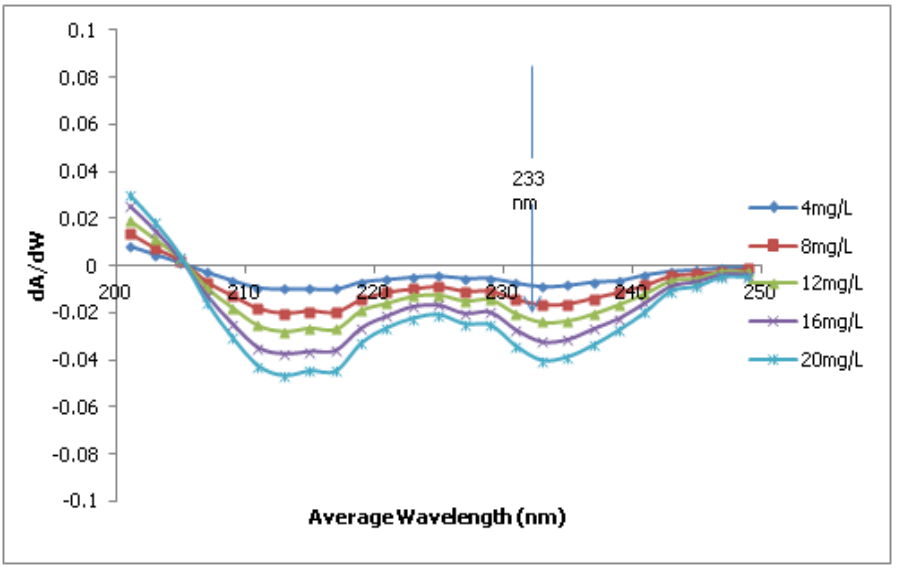

Figure 2. Losartan potassium first-order absorption spectrum at 10 mg/L using distilled water as solvent.

Appendix 4. Comparison of the zero-order spectrum of unspiked and spiked solutions

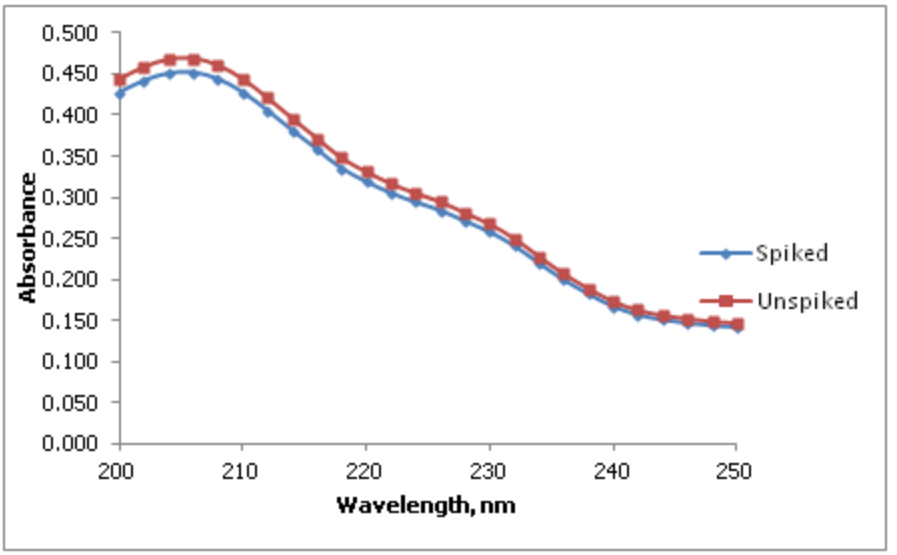

Figure 3. Zero-order absorption spectra of the unspiked and spiked Losartan potassium aqueous solution at 5 mg/L (n=1).

Appendix 5. Comparison of the first-order spectrum of unspiked and spiked solutions

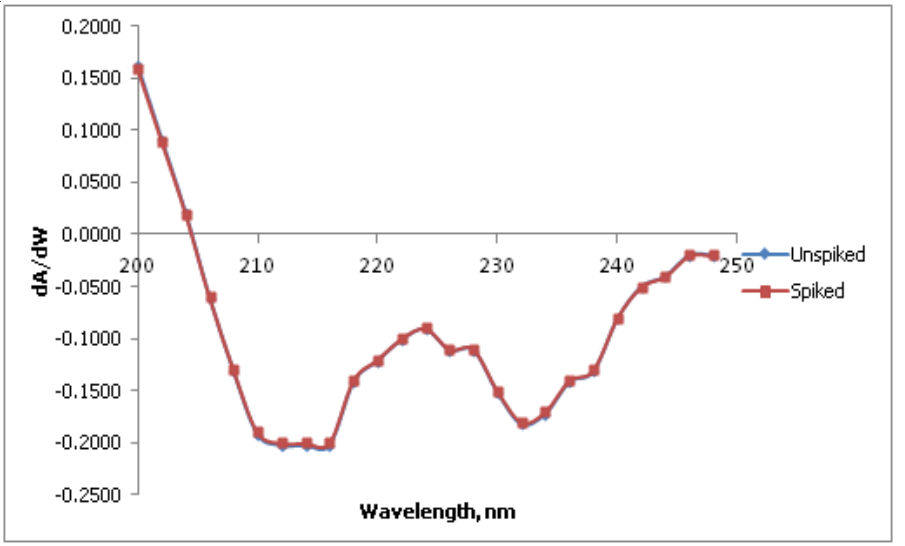

Figure 4. First-order absorption spectra of the spiked and unspiked losartan potassium aqueous solution at 10 mg/L (n=1).

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REFERENCES

Bonfilio R, Favoretto LB, Pereira GR, de Cassia R, Azevedo P, de Araujo M. 2010. Comparative study of analytical methods by direct and first-derivative UV spectrophotometry for evaluation of Losartan potassium in capsules. Brazilian Journal of Pharmaceutical Sciences 46(1): 147-155.

Bryan PD. 2009. What acceptance criteria and specifications should be used for non-clinical dose formulation analysis (NCFDA)? Available from: http://mediaserver.aapspharmaceutica.com/meetings/09AM/Slides/11.12.09_Thu/406%20AB/0700/Peter%20Bryan.pdf.

Chan CC, Lam H, Lee YC, Zhang X. 2004. Analytical method validation and instrument performance verification. New Jersey: John Wiley & Sons, Inc.

Ermer J, Miller, JH, editors. 2005. Method validation in pharmaceutical analysis. Germany: Wiley-VCH.

(ICH) International Conference on Harmonization. 2006. Validation of analytical procedures: Text and methodology Q2(R1). Available from: http://private.ich.org/MediaServer.jser?@_ID=417&@_MODE=GLB.

Jackson EK, Brunton LL, Lazo JS, Parker KL. 2006. Renin and angiotensin. In: Goodman and Gilman's The pharmacological basis of therapeutics, 11th ed. New York: McGraw-Hill Companies, Inc. p 810, 813-814.

(MIMS) Medical Information Management System. 2014. Concise prescribing information of Cozaar. Available from: http://www.mims.com/PHILIPPINES/drug/info/Cozaar/.

Merck & Co., Inc. 2010. Cozaar: Prescribing information, June 2010 ed. NJ: Merck & Co., Inc.

Merck & Co., Inc. 2010.The Merck index, 13th ed. [CD-ROM]. NJ: Cambridge Soft.

Rowe RC, Sheskey PJ, Owen SC, American Pharmacists Association. 2006. Handbook of pharmaceutical excipients. London: Pharmaceutical Press.

Schmauser B. 2008. Pharmaceutical development with focus on paediatric formulations. Available from: http://apps.who.int/prequal/trainingresources/pq_pres/workshop_India-AprilMay08/Day_3/Analy_Meth_Dev.ppt.

Wells, BG, DiPiro, Jt, Schwinghammer, TL, DiPiro, CV. 2009. Pharmacotherapy Handbook, 7th ed. New York: McGraw-Hill Companies, Inc. p 111.

(WHO) The World Health Organization. 2010. WHO Western Pacific Region – Philippines – Health situation and trends. Available from: http://www.wpro.who.int/countries/2010/phl/health_situation.htm.

Youssef NY, Taha EA. 2007. Development and validation of spectrophotometric, TLC and HPLC methods for the determination of lamotrigine in presence of its impurity. Chemistry and Pharmacy Bulletin 55(4): 541-545.

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