第35卷,第12期
2015年12月

光 谱 学 与 光 谱 分 析
Spectroscopy and Spectral Analysis

Vol. 35, No. 12, pp -
December, 2015

Spectrophotometeric Methods for Simultaneous Determination of Amlodipine Besylate and Atenolol in Their Tablet Dosage Form

Nesrine T. Lamie

Analytical Chemistry Department, Faculty of Pharmacy, Cairo University, Kasr El-Aini Street, ET 11562, Cairo-Egypt

Abstract Three simple, specific, accurate and precise spectrophotometric methods are developed for simultaneous determination of amlodipine besylate (AM) and atenolol (AT) in tablets. The first method is dual wavelength spectrophotometry (DW). The second method is ratio subtraction (RS) which depends on subtraction of the plateau values from the ratio spectrum, coupled to first derivative of ratio spectra (^{1}DD). The third method applies bivariate calibration method using 210 and 225 nm as an optimum pair of wavelength for amlodipine and atenolol. The calibration curves are linear over the concentration range of 4～40 $\mu g \cdot mL^{-1}$ for both drugs. The specificity of the developed methods is investigated by analyzing laboratory prepared mixtures of the two drugs and their combined dosage form. The two methods are validated as per ICH guidelines and can be applied for routine quality control testing.

Keywords Amlodipine; Atenolol; Ratio subtraction; Dual wavelength; Derivative-ratio; Bivariate; Spectrophotometry

中图分类号: **文献标识码:** A **DOI:** 10.3964/j.issn.1000-0593(2015)12-00-06

Introduction

Amlodipine (AM) is 2-[(2-aminoethoxy) methyl]-4-(2-chlorophenyl)-1, 4-dihydro-6-methyl-3, 5-pyridine carboxylic acid 3-ethyl5-methyl ester)[1][Fig. 1(a)]. It is a dihydropyridine derivative with calcium antagonist activity. It is used in the treatment of hypertension and chronic stable angina pectoris[2]. It inhibits the transmembrane influx of calcium ions into vascular smooth muscle and cardiac muscle[3]. AM is official in British Pharmacopoeia[4] and United State Pharmacopeia[5] where it is determined by reversed phase high performance liquid chromatographic method, Also UV-spectroscopy method[6] is reported.

Atenolol (AT) is chemically 2-[4-{(2RS)-2- hydroxy-3-[(1-methylethyl) amino] propoxy] phenyl] acetamide[1][Fig. 1(b)]. It is a β-adrenoreceptor blocking agent primarily used for hypertension, angina pectoris and myocardial infarction. It mainly acts by inhibition of renin release, angiotensin-Ⅱ (AT-Ⅱ) and aldosterone production[3]. The British[4] and European Pharmacopoeia[7] describe non-aqueous titration method for the assay of atenolol.

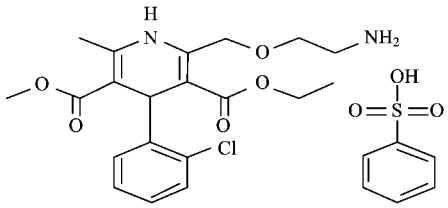

Fig. 1(a) Structural formula for Amlodipine besylate

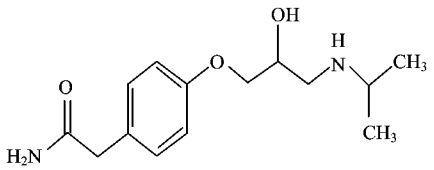

Fig. 1(b) Structural formula for Atenolol

Few methods are available for the simultaneous determination of AM and AT in combination such as RP-HPLC[8], HPTLC[9] and spectrophotometry[10]. The aim of this work is

Received: 2015—; **accepted**: 2015—

Corresponding author e-mail: nesrinelamie@hotmail.com; nesrine.rezkalla@pharma.cu.edu.eg

to develop new, sensitive, accurate, reliable, fast and inexpensive analytical methods for the determination of both drugs without prior separation.

1 Experimental

1.1 Instruments

Spectrophotometer: Shimadzu UV-1650 PC, dual-beam UV-visible spectrophotometer (Japan), with matched 1-cm quartz cells, connected to an IBM-compatible PC and an HP-600 inkjet printer. Bundled, UV-PC personal spectroscopy software Version 3.7 is used to process the absorption and the derivative spectra. The spectral bandwidth is 2 nm with wavelength-scanning speed of 2 800 nm·min^{-1}.

1.2 Materials and reagents

Pure samples are kindly supplied by Epico Pharmaceutical Industry, Cairo, Egypt. AM purity is found to be (100.56±0.945) by high performance liquid chromatographic method[4] while AT purity is (100.96±1.210) by non aqueous titration method[4].

Pharmaceutical formulations

Amlokind-AT tablet dosage forms; labeled to contain 5 mg AM/50 mg AT; batch number A2AFN160 manufactured by Mankind Pharma, India. They are procured from Indian market. Methanol: Spectroscopy grade (E. Merck, Darmstadt, Germany).

1.3 Standard solutions

AM and AT standard solutions (each, 0.1 mg·mL^{-1}), are prepared by dissolving 10 mg of AM and AT, separately in methanol into two 100-mL volumetric flasks and then completing to volume with the same solvent.

1.4 Procedures

1.4.1 Spectral characteristics of AM and AT

The absorption spectra of the two drugs are recorded over the range 200～400 nm using methanol as a blank.

1.4.2 Linearity and construction of calibration curves

1.4.2.1 For Dual wavelength method (DW)

Calibration curves are constructed between the difference in absorbance at 241, 269 and 225, 244 nm for AM and AT, respectively against their corresponding concentrations. The regression equations for both drugs are computed.

1.4.2.2 Ratio subtraction coupled to derivative ratio method (^{1}DD)

Ratio subtraction; [11] for the determination of AT; calibration curve is constructed relating the absorbance of zero order spectra of AT at 226 nm to the corresponding concentrations and the regression equation is computed.

(^{1}DD) method; for the determination of AM in presence of AT, the stored spectra of AM are divided by the spectrum of 24 μg·mL^{-1} AT, then the first derivative of the ratio spectra (^{1}DD) with $\Delta\lambda=4$ nm and a scaling factor=1 is obtained. The amplitude of the first derivative peak of AM/AT is measured at 244.6 nm. A calibration graph relating the peak amplitude at 244.6 nm to the corresponding concentrations of AM is constructed.

1.4.2.3 Bivariate method

This method is based on the simple mathematic algorithm[12], in which data are used from four linear regression equations, two calibrations for each component at two selected wavelengths using the method of Kaiser[13]. The absorbance of pure AM (A) and AT (B) is measured at 210 (λ_1) and 225 (λ_2) nm and then the corresponding regression equations are computed at the selected wavelengths for both AM and AT. The concentrations of AM and AT are calculated using the parameters of the linear regression functions evaluated individually for each component at the same wavelength and substituting in the following equations

$$c_{AM}=\frac{m_{A2}(A_{AB1}-e_{AB1})+m_{A1}(e_{AB2}-A_{AB2})}{m_{A2}m_{B1}-m_{A1}m_{B2}}$$

$$c_{AT}=\frac{A_{AB1}-e_{AB1}-m_{B1}c_{AM}}{m_{A1}}$$

where A_{AB1} and A_{AB2} are the absorbance's of A and B at λ_1 and λ_2, respectively, e_{AB1} and e_{AB2} the sum of the intercepts of the linear calibration at two, wavelengths λ_1 and λ_2 ($e_{AB1}=e_{A1}+e_{B1}$), m_A and m_B the slopes of linear regression and c is the concentrations in μg·mL^{-1}.

1.4.3 Application of the DW, RS, ^{1}DD and bivariate methods for the determination of AM and AT in laboratory prepared mixtures

Into a series of 10-mL volumetric flasks, aliquots equivalent to 40～400 μg·mL^{-1} of AM and of AT are accurately transferred from their standard solutions (each, 0.1 mg·mL^{-1}) with different ratios of the two drugs and the volume is completed with methanol. The spectra of the prepared mixtures are scanned from 200 to 400 nm and stored in the computer.

For Ratio Subtraction Method. The stored zero order absorption spectra of the laboratory prepared mixtures are divided by the absorption spectrum of standard AM′ (24 μg·mL^{-1}), then the amplitudes in the plateau region at 350～380 nm (the constant) is recorded and subtracted from the obtained ratio spectra respectively. Then by multiplying the obtained ratio spectra by AM′ (24 μg·mL^{-1}) to get the zero spectra of AT and the concentrations of AT are calculated using the corresponding regression equation at its λ_{max}.

For dual wavelength and derivative ratio method "as described under calibration curves".

1.4.4 Application to pharmaceutical preparations

To determine the content of AM and AT in commercial tablets (each tablet labeled to contain 5 mg AM and 50 mg

AT, 20 tablets are weighed and finely powdered. A portion of powder equivalent to one tablet is weighed, dissolved in methanol by shaking in ultrasonic bath for about 30 min. The solutions are filtered, transferred quantitatively into 100-mL volumetric flask and completed to the mark with methanol. Transfer 0.8 mL (claimed to contain 40 μg for AM and 400 μg for AT) of this solution into 10 mL volumetric flask and the volume is completed to the mark with methanol. The general procedure described above under each method is followed to determine the concentration of both drugs in the prepared dosage form solution.

2 Results and discussion

The proposed methods can be applied for resolving absorption spectra of two components with high degree of overlap as observed in the wavelength region of 200～300 nm of AM and AT (Fig. 2). This high degree of overlap prevent direct determination of AT.

2.1 Dual wavelength method (DW)

For the determination of AM two wavelengths 241 and 269 nm are selected where the absorbance difference between the two wavelengths is directly proportional to the concentration of AM and the absorbance difference of AT at these two wavelengths is zero. Two wavelengths 225 and 244 nm are selected for the determination of AT where the absorbance difference between them is directly proportional to its concentration and the absorbance difference of AM at these two wavelengths is zero (Fig. 2).

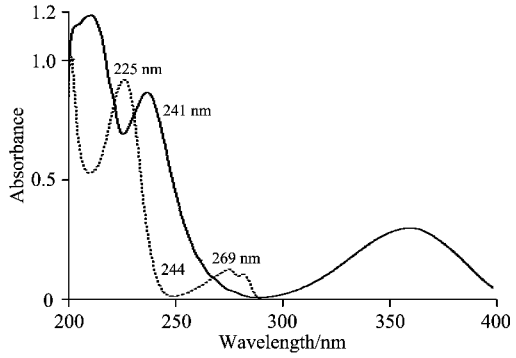

Fig. 2　Absorption spectra og AM 24 μg · mL^{-1} (—) and AT 24 μg · mL^{-1} (--) using methanol as a blank

Difference in absorbances between the selected wavelengths are plotted against corresponding concentration.

The concentrations of AM and AT can be calculated from the following regression equations

$$A_{AM} = 0.03c - 0.001\ 2 \quad r = 0.999\ 5$$

$$A_{AT} = 0.036c + 0.000\ 5 \quad r = 0.999\ 9$$

Where A is the absorbance difference, c is concentration (μg · mL^{-1}) and r is the correlation coefficient.

2.2 Ratio subtraction method coupled to ^{1}DD method

The RS depends on that, when a mixture of AM and AT, where the spectrum of AM is more extended (Fig. 2), the! determination of AT in the mixture can be done by scanning the zero order absorption spectra of the laboratory-prepared mixtures, dividing them by a carefully chosen concentration of standard AM′ (24μg · mL^{-1}) as a divisor. This chosen concentration of the divisor gives the best regression over the proposed concentration range. This will produce new ratio spectra which represent AT/AM′ + *constant* as shown in Fig. 3.

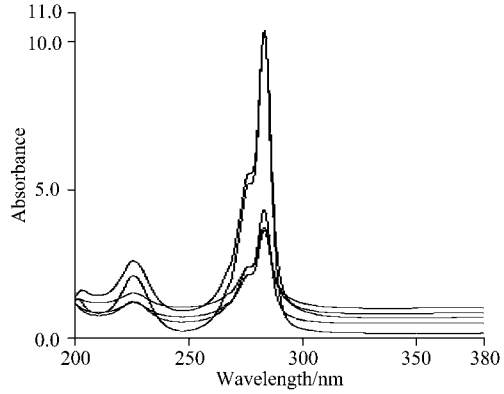

Fig. 3　Ratio spectra of laboratory prepared mixtures of AT and AM using 24 μg · mL^{-1} of AM′ as a divisor and methanol as a blank

Next, subtraction of the values of these constants AM/AM′ in the plateau region (350～380 nm) is done, as shown in Fig. 4. This is followed by multiplication of the obtained spectra by the divisor AM′ (24 μg · mL^{-1}) as shown in Fig. 5, which corresponds to the original spectra of AT.

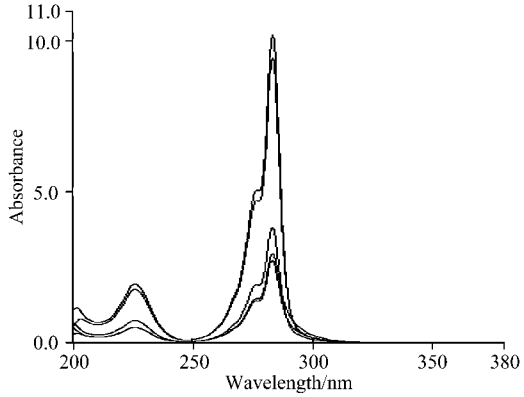

Fig. 4　Ratio spectra of laboratory prepared mixtures of AT and AM using 24 μg · mL^{-1} of AM as a divisor and methanol as a blank after subtraction of the constant

These obtained spectra are used for the direct determination of AT at 226 nm and calculation of the concentration from the corresponding regression equation (obtained by plotting

the absorbance values of the zero order curves of AT at 226 nm against the corresponding concentrations). The regression equation is:

$$A_{AT} = 0.038\ 5c - 0.012\ 2 \quad r = 0.999\ 9$$

Where A is the absorbance, c is concentration ($\mu g \cdot mL^{-1}$) and r is the correlation coefficient. For determination of AM with ^1DD method; the absorption spectrum of the mixture (absorbance at each wavelength) is divided by the absorption spectrum of a standard solution of 24 $\mu g \cdot mL^{-1}$ AT as a divisor Fig. 6.

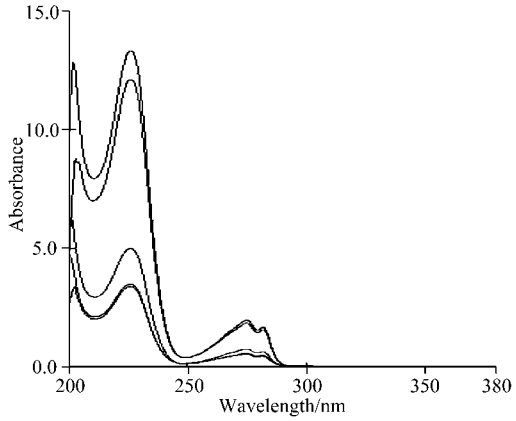

Fig. 5 The zero order absorption spectra of AT obtained by RS for the analysis of laboratory prepared mixtures after multiplication by the divisor AM

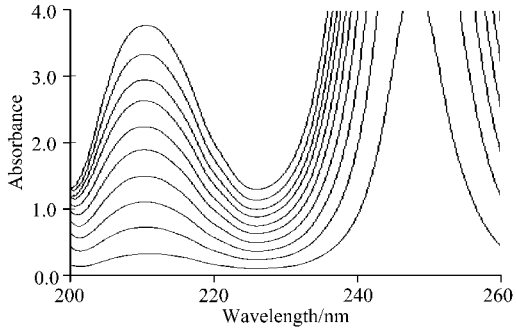

Fig. 6 Ratio spectra of AM (4～40 μg · mL^{-1}) using 24 μg · mL^{-1} of AT as a divisor

Then the first derivative of the ratio spectra is obtained. Fig. 7.

Linear calibration graphs were obtained in concentration range of 4～40 $\mu g \cdot mL^{-1}$ by recording the peak amplitudes at 244.6 nm. The regression equations are computed and found to be:

$$^1DD_{AM} = 0.157\ 4c - 0.041\ 0 \quad r = 0.999\ 6$$

where ^1DD is the peak amplitude of the first derivative of ratio spectra, c is the concentration in $\mu g \cdot mL^{-1}$ and r is the correlation coefficient.

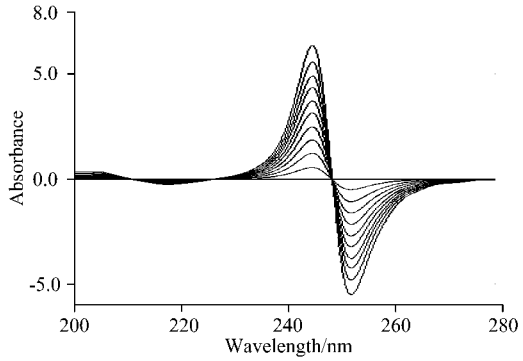

Fig. 7 First derivative of ratio spectra of AM (4～40 μg · mL^{-1}) using 24 μg · mL^{-1} of AT as a divisor and methanol as blank

2.3 Bivariate method

Seven wavelengths are chosen and the slope values of the linear regression equations are estimated for the respective components at the selected wavelengths. Using the obtained data, the sensitivity matrices are created and the respective determinants, proposed by Kaiser's method are calculated (Table 1).

Table 1 Application of Kaiser's method in the selection of wavelength pair for the mixture of AM and AT: the absolute values of determinants of sensitivity matrices (K×10^{-4})

λ/λ	210	215	220	225	230	235	240
210	0	1.35	7.09	11.34	10.14	0.98	5.03
215		0	5.77	10.91	8.84	0.004	5.74
220			0	5.06	3.14	4.42	8.88
225				0	1.81	8.33	11.13
230					0	6.75	10.46
235						0	4.36
240							0

For Bivariate determination of AM and AT, 210 and 225 nm are found to be the optimum pair of wavelength. At these two selected wavelengths, the calibration curves of each drug are obtained in the range of 4～40 $\mu g \cdot mL^{-1}$. The linear regression equations are:

For AM:

$$A_{210} = 0.049\ 2c - 0.002\ 3 \quad r = 0.999\ 8$$
$$A_{225} = 0.029\ 8c - 0.001\ 81 \quad r = 0.999\ 6$$

For AT:

$$A_{210} = 0.023\ 7c - 0.043\ 9 \quad r = 0.999\ 6$$
$$A_{225} = 0.037\ 4c + 0.001\ 2 \quad r = 0.999\ 6$$

where A is the absorbance values, c is the concentration in $\mu g \cdot mL^{-1}$ and r is the correlation coefficient.

For all the proposed methods, the statistical parameters of the regression equations and the concentration ranges are

shown in (Table 2). The selectivity of the proposed procedures is assessed by the analysis of laboratory prepared mixtures containing different ratios of AM and AT where satisfactory results are obtained over the calibration ranges as shown in Table 2.

The proposed methods are applied for the determination of AM and AT in their combined pharmaceutical formulation (amlokind tablets) and the validity is further assessed by applying the standard addition technique (Table 3).

Table 2　Assay validation parameters of the proposed spectrophotometric methods for the determination of pure samples of AM and AT

Parameter	DW		RS	^{1}DD	Bivariate	
	AM	AT	AT	AM	AM	AT
Accuracy (mean±S. D.)	100. 05±1. 109	100. 14±0. 762	100. 07±1. 007	99. 99±1. 185	99. 79±1. 171	100. 30±0. 995
Specificity*	100. 75±1. 112	99. 86±0. 924	100. 33±1. 250	99. 53±0. 864	100. 87±1. 055	99. 21±1. 059
Precision Repeatability**	0. 984	0. 801	0. 490	0. 965	1. 208	0. 719
Intermediate precision***	1. 133	1. 186	1. 017	1. 163	0. 954	0. 822
Linear range/(μg·mL^{-1})	4～40	4～40	4～40	4～40	4～40	4～40
Slope	0. 030	0. 036	0. 039	0. 157	0. 049	0. 037
Standard error of the Slope	0. 000 3	0. 000 1	0. 000 1	0. 001 6	0. 000 4	0. 000 37
Intercept	−0. 001 2	0. 000 5	−0. 012 2	−0. 041 0	−0. 002 3	0. 001 2
Standard error of the intercept	0. 007 8	0. 002 5	0. 003 0	0. 038 7	0. 008 8	0. 009 1
Correlation (r) coefficient	0. 999 5	0. 999 9	0. 999 9	0. 999 6	0. 999 8	0. 999 6

* Laboratory prepared mixtures;

** The intraday (n=3) relative standard deviations of 12, 16 and 20 μg·mL^{-1} of AM and AT by the proposed methods;

*** The interday (n=3) relative standard deviations of 12, 16 and 20 μg·mL^{-1} of AM and AT by the proposed methods.

Table 3　Quantitative determination of AM and AT in Amlokind-AT tablets by the proposed spectrophotometric methods and results of application of standard addition technique

Amlokind-AT tablets Batch No. A2AFN160 5 mg AM and 50 mg AT	DW	RS	^{1}DD	Bivariate
AM found%±S. D. *	101. 25±0. 683		100. 33±0. 91	100. 22±0. 693
Standard addition	99. 17±1. 101		101. 08±1. 28	100. 60±1. 185
AT found%±S. D. *	100. 49±0. 910	98. 96±0. 974		99. 42±0. 592
Standard addition	101. 33±0. 572	99. 29±1. 230		100. 55±0. 396

* Average of three different determinations.

2. 4　Statistical analysis

Results of the suggested methods for determination of AM and AT are statistically compared with those obtained by applying official methods (Table 4). The calculated t- and F-values[14] are found to be less than the corresponding theoretical ones, confirming good accuracy and excellent precision.

Table 4　Statistical analysis of the results obtained by the proposed spectrophotometric methods and official methods for the determination of AM and AT in pure powder form

Parameter	AM				AT			
	Official* method	DW	^{1}DD	Bivariate	Official** method	DW	RS	Bivariate
Mean	100. 56	100. 05	99. 99	99. 79	100. 96	100. 14	100. 07	100. 30
S. D.	0. 945	1. 109	1. 185	1. 171	1. 210	0. 762	1. 007	0. 995
Variance	0. 893	1. 230	1. 404	1. 371	1. 464	0. 581	1. 014	0. 990
n	6	10	10	10	6	10	10	10
Student's t test (2. 145)		0. 936	0. 998	1. 360		1. 676	1. 589	1. 185
F value		1. 377 (4. 77)	1. 572 (4. 77)	1. 535 (4. 77)		2. 520 (3. 48)	1. 444 (3. 48)	1. 479 (3. 48)

* HPLC method using octadecylsilyl silica gel (5 μm) (150 mm, 3. 9 mm i. d.), mobile phase: 15 mL acetonitrile: 35 mL methanol: 50 mL buffer solution, flow rate: 1 mL·min^{-1}, UV detection: 237 nm;

** Non aqueous titration method;

The values in parenthesis are the corresponding tabulated t and Fvalues at P=0. 05.

3 Conclusion

From the previous discussion, it can be concluded that the proposed procedures are simple, do not require sophisticated techniques or instruments. They are also sensitive and selective and can be used for the routine analysis of AM and AT in their available dosage form. The methods are also suitable and valid for application in laboratories lacking liquid chromatographic instruments.

References

[1] The Merck Index, Merck Research Laboratories; white house station, 14th ed., 2006.
[2] McEvoy G K. American Hospital Formulary Service American Society of Health-System Pharmacists Inc., Bethesda, 2001.
[3] Sweetman S C. Martindale the Complete Drug Reference, 34th ed., Pharmaceutical Press, London, 2005. 862.
[4] The British Pharmacopoeia. Her Majesty's Stationery Office, London, 2010.
[5] Rockville M D. The United State Pharmacopoeia, USP28-NF23 ed., United States Pharmacopoeial Convention Inc., 2009.
[6] Audhari B G. AB Patel. Int. J Chem Tech Res., 2010, 633.
[7] European Pharmacopoeia, 2nd Ed., Council of Europe, 1995.
[8] Barman R K, Islam M A, Ahmed M, et al. J. Pharm. Sci., 2007, 20(4): 274.
[9] Argekar A P, Powar S G. J. Pharm. Biomed. Anal., 2000, 21(6): 1137.
[10] Rathee P, Rathee S, Thakur S, et al. Int. J. of Chem. Tech. Research 2010, 2(1): 62.
[11] El-Bardicy M G, Lotfy H M, El-Sayed M A, et al. J AOAC Int., 2008, 91: 299.
[12] Lopez-de-Alba P, López-Martínez L, Wrobel-Kaczmarczyk K, et al. Anal. Lett., 1996, 29: 487.
[13] Massar D L, Vandeginste B G M, Deming S N, et al. Chemometrics: A Textbook, Elsevier, Amsterdam, 1988. 124.
[14] Spiegel M R, Stephens L J. Schaum Outline of Theory and Problems of Statistics, Schaum Outline Series, New York, 1999.