Spectrophotometric and Reversed-Phase High-Performance Liquid Chromatographic Methods for Simultaneous Determination of Paracetamol and Balsalazide Disodium Dihydrate in Combined Tablet Dosage Form

Mayank Mandhanya*[1], Nitin Dubey [1], Nidhi Dubey [2], Praveen Sharma [1], Shiv Shankar Hardenia [1],

[1] College of Pharmacy, IPS Academy, Indore [Madhya Pradesh], India

[2] School of Pharmacy, DAVV, Indore [Madhya Pradesh], India

*Corresponding author E-mail: mayankmandhanya@gmail.com Phone#:0731-2381313, 91-9424583386

Abstract:

Simple, accurate, precise and sensitive ultraviolet spectrophotometric and reversed-phase high-performance liquid chromatographic (RP-HPLC) methods for simultaneous estimation of paracetamol (PCM) and Balsalazide Disodium Dihydrate (BAL) in combined tablet dosage form have been developed and validated. Linearity of PCM and BAL was found in concentration range of 2-30 and 1–12 µg/mL in methanol at 249 and 230 nm, respectively for spectroscopic method. Instrument used for RP-HPLC method was Shimadzu LC 10 AT VP system with Luna C18 column and methanol: acetonitrile: water (55:30:15 v/v/v) as the mobile phase. The detection was carried out using a diode array detector set at 239 nm. Linearity of the LC method was in the concentration range of 5.0–100.0 and 5-60 µg/mL for PCM and BAL respectively. The recoveries were in the range of 99.70 ± 0.38 and 99.38 ± 0.36 for PCM and 99.85 ± 0.09 and 99.31 ± 0.23 for BAL in simultaneous equation method and HPLC method respectively. Both methods have been successfully applied for the analysis of the drugs in a pharmaceutical formulation. Results of analysis were validated statistically.

Key words: Absorptivity, robustness, simultaneous equation, validation

---

Introduction:

Paracetamol (PCM) is chemically known as N-(4-hydroxyphenyl)acetamide and belongs to the class of compounds known as NSAID (British pharmacopoeia 2004; United state pharmacopoeia 2007; Indian pharmacopoeia 1998) and also having antipyretic action. It is official in Indian Pharmacopoeia, British Pharmacopoeia and United States Pharmacopoeia. Its action mediates through cyclo-oxygenase-3 inhibition and modulation of central serotonergic pathways. Balsalazide Disodium Dihydrate (BAL) is chemically known as (E)-5-[[-4-[[(2- carboxyethyl) amino] carbonyl] phenyl]azo]-2-hydroxybenzoic acid and is official in British Pharmacopoeia (2004), United state pharmacopoeia 30-National formulary 25(USP30-NF25) BAL is NSAID with prominent analgesic and has been most effective for the treatment of rheumatoid arthritis (British pharmacopoeia 2004; United state pharmacopoeia 2007). Chemical structures of PCM and BAL are shown in Fig. 1.

Literature survey revealed that the assay of the PCM in pure and dosage forms is official in Indian Pharmacopoeia , British Pharmacopoeia and USP30-NF25 (British pharmacopoeia 2004; United state pharmacopoeia 2007; Indian pharmacopoeia 1998) apart from Pharmacopeias several analytical methods have been reported for the determination of PCM in biological fluids and urine (Nicholls *et al*. 1997; Hart *et al*.1984; Hewavitharana *et al*. 2008) including column high-performance liquid chromatography (HPLC), HPLC/MS, and HPLC-NMR. HPLC method for determination of BAL from tablet formulation is official in USP30-NF25 and BP (2004). Several analytical methods that have been reported for the determination of BAL in biological fluids and in bulk as well as pharmaceutical formulations include HPLC, UV absorption spectrophotometry, fluorometry, gas chromatography/MS (GC/MS), and Fourier transform Raman and infrared spectrophotometry (Nobilis M e*t al*. 2004; Kobylinska *et al.* 2003; Margarita *et al*. 2003).

This paper describes simple, accurate, precise, and sensitive UV-spectrophotometric and reversed-phase RP-HPLC methods for simultaneous determination of PCM and BAL in a combined tablet dosage form. The proposed methods were optimized and validated according to International Conference on Harmonization (ICH) guidelines.

Materials and Methods:

Drugs and Chemicals:

Acetonitrile (HPLC grade) and methanol (HPLC and AR grade) were purchased from Merck (Mumbai, India) and water (HPLC grade and AR grade) was prepared in institute. All other reagents used were of analytical grade for the spectrophotometric determination and of HPLC grade for the HPLC method. Standard bulk drug samples of PCM (99.60% pure) and BAL (99.80% pure) were provided by Ipca laboratories limited (Ratlam, India) as gratis sample. The pharmaceutical dosage form used in this study was Nilitis-P tablets labeled to contain PCM 500 mg and BAL 500 mg/tablet (Ipca lab, Mumbai, India).

Instrumental:

A UV-visible double beam spectrophotometer (Model 1601; Shimadzu, Japan) with 1 cm matched quartz cells and UV probe software version 2.10 was used for the spectrophotometric method. For the HPLC method, an HPLC system consisting of LC 10 AT VP pump equipped with diode array detector (Shimadzu, Japan) and Luna C18 (4.6 mm id) column and class M10A software version 1.6 was used. A Rheodyne (Rohnert Park, CA) injector with 20 µL loop was used for injecting the sample.

Method I – Spectroscopic method (Simultaneous equation method) (Gandhi *et al*. 2008)

A stock solution of each drug having a concentration of 1 mg/mL (i.e.1000 µg/mL) was prepared by dissolving PCM and BAL separately in methanol. Aliquots of the stock solutions were further diluted in distilled water and were scanned in the wavelength range of 400–200 nm. Zero order overlain spectra are presented in Fig. 2. The determinations were carried out at 249 and 230 nm, the maximum absorbance wavelength (λ_{max}) of PCM and BAL, respectively. Appropriate dilution were prepared using water from the stock solution of 1000 µg/mL of PCM and BAL to get aliquots of the concentration of 2, 4, 6, 8 and 10 µg/mL. The calibration curves were plotted from mean absorbance values of observation of the six replicate. The absorptivity values for both the drugs were determined at their respective λ_{max} by measuring absorbance values for working standards of PCM and BAL.

Procedure for analysis of tablet formulation - Twenty tablets were weighed accurately, and a quantity of tablet powder equivalent to 100 mg PCM and 100 mg BAL was transferred to a 100 mL volumetric flask, 70 mL methanol was added, and the flask was shaken vigorously for 5 min. The volume was made up to the mark with methanol. The solution was filtered and further diluted with distilled water to obtain a concentration within the Beer's law range. The absorbance of sample was measured at 249 and 230 nm. The contents of PCM and BAL were calculated by solving the following equations.

$$A_1 = a_{x1}.b.C_x + a_{y1}.b.C_y$$

$$A_2 = a_{x2}.b.C_x + a_{y2}.b.C_y$$

Where, a_{y1} and a_{y2} are the absorptivity of drug Y at λ_1 and λ_2,

A_1 and A_2 are the absorbencies of the diluted sample at λ_1 and λ_2,

b is the path length and

C_x and C_y is the concentration of PCM and BAL respectively in diluted sample.

Method II – Chromatographic method (RP-HPLC method) (Gopinath *et al.* 2007; Gandhi *et al*. 2008; Sabnis *et al*. 2008)

In the RP-HPLC method, separation and analysis of PCM and BAL were carried out on a Luna C18 column (4.6 mm id) with the diode array detector set at 239 nm. Mobile phase consisting of methanol: acetonitrile: water (55:30:15 v/v/v; filtered through a 0.2 µm membrane filter, degassed and sonicated) was used with a flow rate of 0.6 mL/min.

(a) Standard stock solutions - Standard stock solutions containing 100 µg/mL PCM, 10 µg/mL BAL were prepared by dissolving the pure drugs separately in the mobile phase.

(b) Preparation of the calibration curves - Aliquots of 1, 2, 3, 4, 5 and 6 mL stock solution of PCM and 0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 mL stock solution of BAL were transferred into a series of 10 mL volumetric flasks and the volume was made up to the mark with the mobile phase. Each solution was injected, and chromatogram was recorded. Mean retention times for PCM and BAL were found to be 4.608 and 7.057 min, respectively. The peak area of PCM and BAL were noted, and respective calibration curves were plotted as peak area against concentration of each drug.

(c) Procedure for analysis of tablet formulation - Twenty tablets were weighed accurately and a quantity of tablet powder equivalent to 50 mg PCM and 50 mg BAL weighed and transferred to a 50 mL volumetric flask containing about 35 mL mobile phase, ultrasonicated for 5 min, and the volume was made up to the mark with the mobile phase. The solution was filtered through Whatman (Florham Park, NJ) No. 41 paper, 0.2 mL filtrate was transferred to a 10 mL volumetric flask and the volume was made up to the mark with the mobile phase. The tablet sample solution was injected, the chromatogram was obtained and the peak areas were recorded. A representative chromatogram is given in Fig 3. Form the peak area the both the drugs concentration of each drug/tablet was estimated from the respective calibration curves.

(d) Robustness studies - The influence of small, deliberate variations of the analytical parameters on the retention time of the drugs was examined. The following factors were selected for change: the wavelength at which the drugs were recorded (239 ± 1 nm) and the flow rate of the mobile phase (0.6 ± 0.02 mL/min). One factor at the time was changed to estimate the effect. The solutions containing 20 µg/mL PCM and 20 µg/mL BAL were applied onto the column. Six replicate analyses (n = 6) were conducted at 3 levels of the factor (–, 0, +).

(e) Recovery studies - Accuracy of the method were analyzed by recovery studies carried out by addition of standard drug solution to pre-analyzed sample at 3 different levels: 80, 100, and 120%.

(f) Precision - Precision of the method was checked by 3 replicate readings at 3 concentration levels of within range expressed as RSD values.

Statistical Analysis: The statistical analysis was performed using Microsoft Excel 2003.

Results and Discussion:

For spectroscopic method (Method- I) methanol was used as common solvent for both the drugs. Linearty range of PCM and BAL found in the concentration range of 2-30 and 1-12 µg/mL at 249 nm and 230 nm, respectively.

For the RP-HPLC method (method-II), chromatographic conditions were optimized to achieve the best resolution and peak shape for PCM and BAL. Different mobile phases containing methanol, acetonitrile and water were examined (data not shown), and the mobile phase methanol: acetonitrile: water (55:30:15 v/v/v) was selected as optimal for obtaining well-defined and resolved peaks. The optimum wavelength for detection and quantitation was 239 nm, at which the best detector response for both the substances was obtained.

Straight line calibration curves were obtained for PCM and BAL in method-I and method-II. Table 1 summarizes the Beer's law limit, linear regression equation, correlation coefficient, standard deviations (SD), and limit of

detection (LOD) and limit of quantitation (LOQ) values for both methods all the statistical validation parameters were found to be satisfactory and as per ICH guidelines. System suitability parameters for the RP-HPLC method are listed in Table 2.

Although spectroscopic and chromatographic methods (British pharmacopoeia 2004; Hart *et al.* 1984; Hewavitharana *et al.* 2008; Indian pharmacopoeia 1998; Kobylinska *et al.* 2003 united state pharmacopoeia 2007) have been reported for analysis of PCM and BAL in bulk drug, biological fluid and urine.

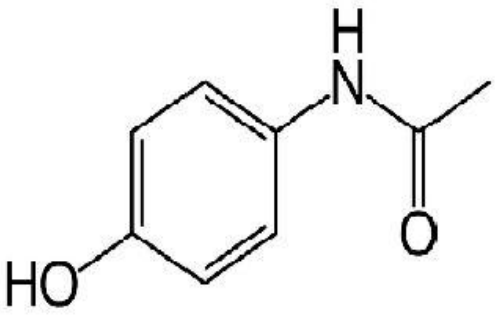
Robustness studies of the HPLC method, carried out after deliberate alterations of the analytical wavelength and flow rate of mobile phase, showed that small changes of these operational parameters did not lead to changes of retention times for the peaks of interest. The effect of a single factor at two levels indicated that the selected factors remained unaffected by small variations of these parameters. Therefore, this method is suitable for routine analysis (Table 3).

The proposed methods were also evaluated in the assay of commercially available tablets containing PCM and BAL. Six replicate determinations were performed on the accurately weighed amounts of tablets. For PCM, recovery (mean, %, ± SD, n = 6) was found to be 99.71 ± 0.31 and 99.88 ± 0.29 for Methods I and II, respectively. For BAL, recovery was found to be 99.05 ± 0.50 and 99.31 ± 0.51 for Methods I and II, respectively (Table 4).

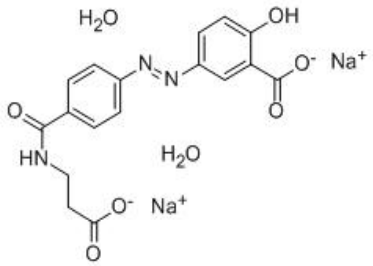
For PCM, the recovery study results ranged from 98.86 to 100.20% and 98.54 to 99.89% for Methods I and II, respectively, with relative standard deviation (RSD) values ranging from 0.001 to 0.005 and 0.004 to 0.005%, respectively. For BAL, the recovery results ranged from 98.93 to 100.23 and 98.67 to 99.89% for Methods I and II, respectively, with RSD values ranging from 0.0003 to 0.007 and 0.005 to 0.004%, respectively. Results of recovery studies are reported in Table 5.

Conclusions:

The validated UV-spectrophotometric and RP-HPLC methods developed here proved to be simple, fast, accurate, precise and sensitive. Thus, they may be used for routine analysis of PCM and BAL in combined tablet dosage form without prior separation.



PARACETAMOL



Balsalazide Disodium Dihydrate

Figure 1. Chemical structures of PCM and BAL.

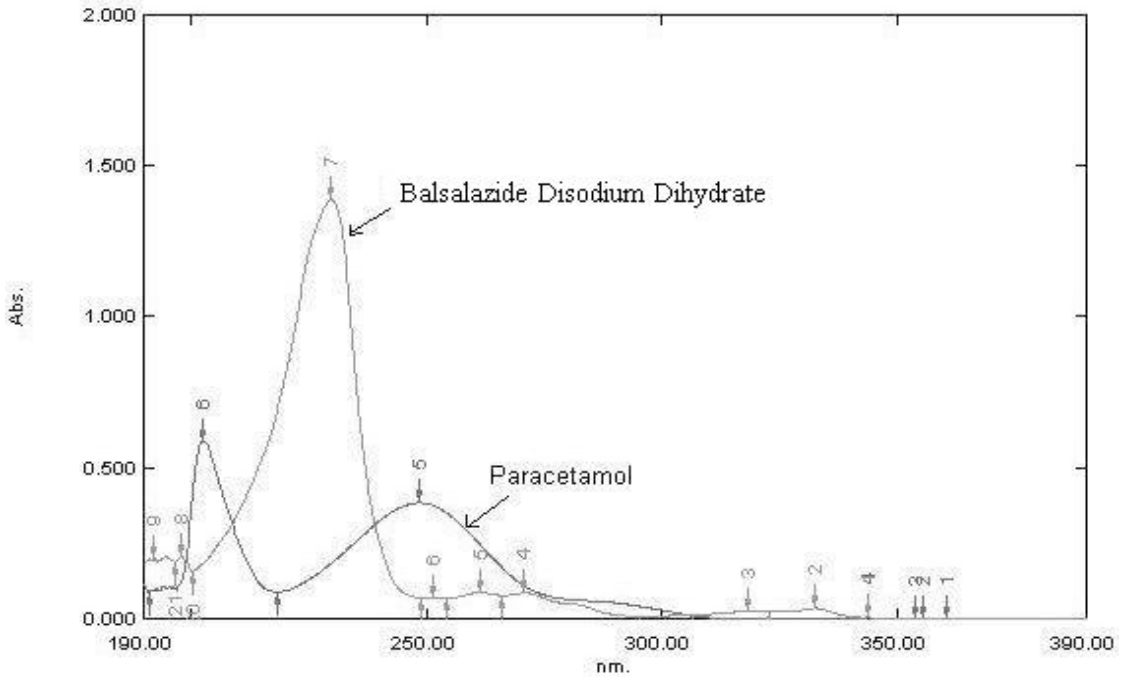


Figure 2. Overlain spectra of PCM (4 µg/mL) and BAL (4 µg/mL).

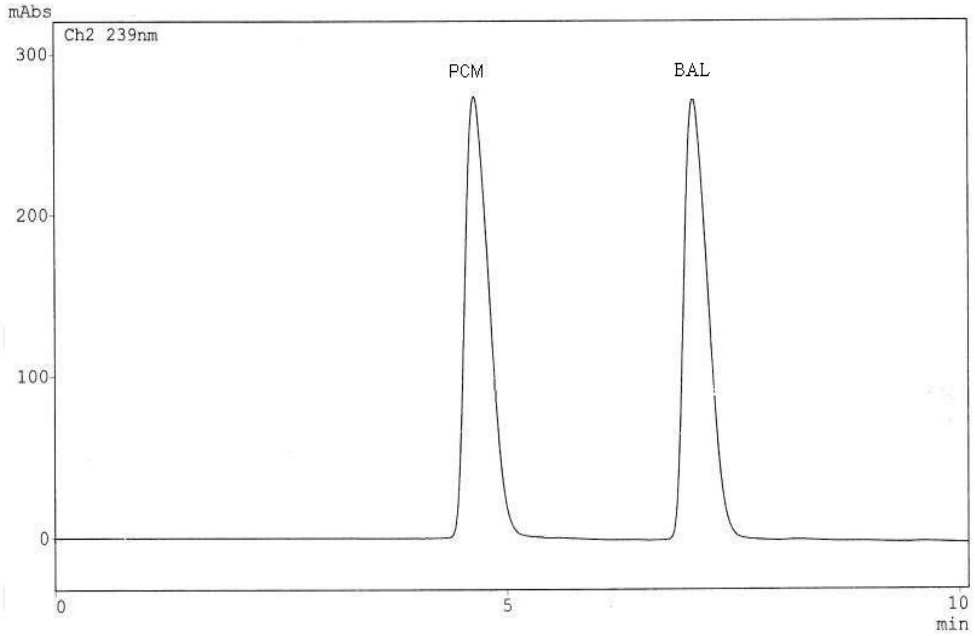


Figure 3. Chromatogram of PCM (20 µg/mL, retention time 4.608 min); BAL (20 µg/mL, retention time 7.057 min).

Table 1. Regression analysis of calibration curves of method I and II.

Parameters	Method I		Method II	
	PCM	BAL	PCM	BAL
λ_{max}	249	230	239[a]	239[a]
Beer's law limit, µg/mL	2-30	1-12	5-100	5-60
Correlation coefficient	0.9994	0.999	0.9992	0.9994
Molar absorptivity	0.094	0.038	-	-
Linear regression equation[b]				
Intercept	0.0041	0.0728	- 22548	21685
Slope	0.919	0.337	204846	236829
SD[c]	0.014	0.027	10053.6	13074.9
Detection limit, µg/mL	0.51	0.27	0.16	0.18
Quantitation limit, µg/mL	1.56	0.82	0.49	0.55

[a] Detection wavelength for HPLC method.
[b] y = mx + c, where y is the absorbance and x is the concentration (µg/mL).
[c] SD = standard deviation.

Table 2. System suitability parameters for RP-HPLC method.

Parameters	Paracetamol	BALumetone
Calibration range, µg/mL	5-100	5-60
Theoretical plate number	2974	3808
HETP[a]	0.0084	0.0066
Asymmetric factor	1.14	0.92
Tailing factor	1.29	1.03
Capacity factor (k')	1.26	3.92
Resolution	-	6.74

[a] HETP = Height equivalent to theoretical plate, cm

Table 3. Robustness data in terms of retention time for PCM and BAL[a].

Level	Wavelength[b]		Flow rate[c]	
	PCM	BAL	PCM	BAL
-	4.608 ± 0.085	7.057 ± 0.152	4.608 ± 0.084	7.057 ± 0.097
0	4.608 ± 0.093	7.057 ± 0.124	4.608 ± 0.082	7.057 ± 0.015
+	4.609 ± 0.110	7.057 ± 0.101	4.608 ± 0.134	7.057 ± 0.059

[a] Mean ± SD, n = 6.
[b] 230 ± 1 nm.
[c] 0.6 ± 0.02 mL/min.

Table 4. Results of analysis of commercial formulation.

Method	Label claim, mg/tablet		% claim, estimated[a]		Standard deviation	
	PCM	BAL	PCM	BAL	PCM	BAL
I	500	500	99.48 ± 0.011	99.89 ± 0.0015	1.1	0.15
II	500	500	100.1 ± 0.0061	99.96 ± 0.0035	0.616	0.351

[a] Average of 6 determinations.

Table 5. Recovery studies of PCM and BAL by Methods I and II.

Drug	Concentration taken, µg/ml for methods	Concentration added, µg/ml for methods	Total concentration found µg/ml		Recovery, %[a]	
			Method I	Method II	Method I	Method II
PCM	5	4	8.94	8.92	99.34 ± 0.0052	99.11 ± 0.0046
	5	5	9.96	9.92	99.68 ± 0.0029	99.25 ± 0.0054
	5	6	11.01	10.86	100.1 ± 0.0011	99.80 ± 0.0050
BAL	5	4	8.99	8.96	99.92 ± 0.0003	99.55 ± 0.0050
	5	5	9.99	9.90	99.98 ± 0.0013	99.29 ± 0.0056
	5	6	10.97	10.90	99.75 ± 0.0071	99.09 ± 0.0049

[a] mean ± relative standard deviation (n = 3).

Acknowledgments:
The authors wish to express their gratitude to Ipca Lab., Ratlam, India, for providing gratis samples of pure PCM and BAL.

References:

British Pharmacopoeia (2004) British Pharmacopoeia Commission Office, London, UK, pp 1343-1344, 1479-1481

Davidson AG (2004) in *Practical Pharmaceutical Chemistry, Part 2,* A.H. Beckett & J.B. Stenlake (Eds), CBS Publishers and Distributors, New Delhi, India, pp 282–283

Gandhi SV, Dhavale ND, Jadhav VY, Sabnis SS (2008) Spectrophotometric and Reversed-Phase High-Performance Liquid Chromatographic Methods for Simultaneous Determination of Escitalopram Oxalate and Clonazepam in Combined Tablet Dosage Form. *Journal of AOAC International* **91**, 33-38

Gopinath R, Rajan S, Meyyanathan SN, Krishnaveni, N, Suresh B (2007) RP-HPLC method for simultaneous estimation of paracetamol and aceclofenac in tablets. *Indian Journal of Pharmaceutical Science* **69**, 137–140

Hart SJ, Aguilar MI, Healey K, Smail MC, Calder IC (1984) Improved high-performance liquid chromatographic separation of urinary paracetamol metabolites using radially compressed columns. *Journal of Chromatography B* **306**, 215-229

Hewavitharana AK, Lee S, Dawson PA, Markovich D, Shaw PN (2008) Development of an HPLC-MS-MS method for the selective determination of paracetamol metabolites in mouse urine. *Analytical Biochemistry* **374**, 106-111

ICH Harmonized Tripartite Guideline (2005) *Validation of Analytical Procedures: Text and Methodology* Q2 (R1), International Conference on Harmonization, Geneva, Switzerland

Indian Pharmacopoeia (1998) Controller of publication, Delhi, pp 554-555

Kobylinska K, Barlinska M, Kobylinska M (2003) Analysis of Balsalazide Disodium Dihydrate in human plasma by HPLC, Application to single dose pharmacokinetic studies. *Journal of Pharmaceutical and Biomedical Analysis* **32,** 223-228

Margarita V, Costs SMB (2003) Photodegradation of Balsalazide Disodium Dihydrate in aqueous solutions. *Journal of Photochemistry and Photobiology A: Chemistry* **157**, 93-101

Nicholls AW, Farrant RD, Shockcor, JP, Unger, SE, Wilson, ID, Lindon, JC, Nicholson, JK (1997) NMR and HPLC-NMR spectroscopic studies of futile deacetylation in paracetamol metabolites in rat and man. *Journal of Pharmaceutical and Biomedical Analysis* 15, 901-910

Nobilis M, Hol MA., Kola LO, Kopecky J, Kune M, Svoboda Z, Kve JT (2004) Identification and determination of phase II Balsalazide Disodium Dihydrate metabolites by high-performance liquid chromatography with photodiode array and mass spectrometric detection. *Journal of Chromatography A* **1031**, 229-236

Sabnis SS, Dhavale ND, Jadhav, VY, Gandhi SV (2008) Column reversed-Phase High-Performance Liquid Chromatographic Method for Simultaneous Determination of Rabeprazole Sodium and Domperidone in Combined Tablet Dosage Form. *Journal of AOAC International* **91**, 344-348

United States Pharmacopeia (2007) The United States Pharmacopeial Convention, Rockville, MD, pp 16-17