

Total polyphenols from *Syzygium cumini* (L.) Skeels fruit extract

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A precise, accurate and low cost spectrophotometric method was developed and validated for routine determination of total polyphenols, as pyrogallol acid equivalents, from the percolated and lyophilized extract of *Syzygium cumini* (L.) Skeels fruits. Validation was assessed experimentally and data were rigorously treated by statistical analysis. Analytical parameters were: linearity, interval (range), precision and recovery/accuracy, limit of detection (LOD, $\mu\text{g mL}^{-1}$) and limit of quantification (LOQ, $\mu\text{g mL}^{-1}$). The visible spectrophotometric method presented linearity ($r^2 = 0.9979 \pm 0.0010$) over the concentration range 0.25-7.5 $\mu\text{g mL}^{-1}$ of standard pyrogallol acid, precision $\leq 2.918171\%$, recovery/accuracy ranging from 96.228693 to 107.17701%, LOD = 0.21 $\mu\text{g mL}^{-1}$ and LOQ = 0.64 $\mu\text{g mL}^{-1}$.

Uniterms: Total polyphenols. *Syzygium cumini*. Botanical extract. Analytical method validation. Spectrophotometry.

Um método espectrofotométrico preciso, rigoroso e de baixo custo foi desenvolvido e validado para a determinação de polifenóis totais, utilizando-se como padrão o ácido pirogálico. O extrato dos frutos de *Syzygium cumini* (L.) Skeels foi preparado empregando-se o método de percolação com posterior liofilização. A validação foi executada experimentalmente e os dados foram submetidos à análise estatística. Os parâmetros analíticos considerados foram: linearidade, intervalos de precisão e de recuperação, limite de detecção (LD, $\mu\text{g.mL}^{-1}$) e o limite de quantificação (LQ, $\mu\text{g.mL}^{-1}$). O método espectrofotométrico apresentou linearidade ($r^2 = 0,9979 + 0,0010$) ao longo do intervalo de concentração de 0,25-7,5 $\mu\text{g mL}^{-1}$ de ácido pirogálico, padrão de precisão menor do que 2,918171%; recuperação/precisão entre 96,228693 a 107,17701%, e LD = 0,21 $\mu\text{g.mL}^{-1}$ e LOQ = 0,64 $\mu\text{g mL}^{-1}$.

Unitermos: Polifenóis. *Syzygium cumini*. Extrato vegetal. Métodos analíticos/validação. Espectrofotometria.

INTRODUCTION

Syzygium cumini (L.) Skeels (Synonym: *S. jambolanum*, *E. jambolana*) (Myrtaceae), popularly known in Brazil as "jambolão" (jambolan or java plum in English), is a native tree of the tropics, originally from India and SE Asia. It is widespread in some states of North, Northeast and Southeast Brazil (Grover *et al.*, 2001; Migliato *et al.*, 2006; Migliato *et al.*, 2007, Rey-

nertson *et al.*, 2008) and is used as a popular treatment against various diseases. In Brazil, the bark, fruits, seeds and leaves of this plant are used for the treatment of diabetes and administered in various pharmaceutical preparations (e.g., aqueous or alcoholic extract, decoctions or crude plant juice) (Braga *et al.*, 2007). *Syzygium cumini* seeds have already shown hypoglycemic and antioxidant activities. A decoction of the bark is also used for dysentery and diarrhea. Moreover, *Syzygium cumini* has been shown to have sedative and anticonvulsant effects and a potent central nervous system depressant effect (Pepato *et al.*, 2004).

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Syzygium species are reported to be very rich in tannins, flavonoids, essential oils, anthocyanins and others phenolic constituents (Sharma *et al.*, 2003; Migliato *et al.*, 2007, Reynertson *et al.*, 2008). Scalbert (1991) reviewed the antimicrobial properties of tannins.

According to Shafi and co-workers (2002), plant extracts of *Syzygium* species have known antibacterial activity. In addition, Chandrasekaran and Venkatesalu (2004) showed that aqueous and methanol extracts inhibited the growth of some of the fungal microorganisms implicated in skin diseases, such as *C. albicans*, *T. rubrum*, *T. mentagrophytes* and *M. gypseum*.

The aim of this research work was to validate a routine and low cost spectrophotometric method (mainly to be used as a quality control tool) to quantify total polyphenols, as pyrogalllic acid equivalents, from the percolated and lyophilized extract of fruits from *Syzygium cumini* (L.) Skeels.

MATERIAL AND METHODS

Solvents, reagents and reference standard

All reagents and solvents were of analytical grade and they were purchased from Merck (Germany), Sigma-Aldrich (Brazil) and LabSynth (Brazil), and used without any further purification (sodium carbonate PA-ACS, ethanol 99.5%, Folin-Ciocalteu 2 N). Pyrogalllic acid $\geq 99\%$, from Riedel-de Haën (Sigma-Aldrich) was used as reference standard.

Plant Material

The fruits of *Syzygium cumini* (L) Skeels were collected during December 2006 to January 2007, in the Medicinal and Toxic Plant Garden of FCF/UNESP, Araraquara – SP, Brazil. On the same opportunity, the voucher was deposited under the number 19586 in SJRP Herbarium, Department of Botany of IBILCE-UNESP of São Jose do Rio Preto Campus, by Dr. Neusa Taroda Ranga.

Preparation of *Syzygium cumini* (L.) Skeels extract

Extract was obtained by percolation using *Syzygium cumini* fruits. The extract was prepared with 10% w/v of *Syzygium cumini* fruits in ethanol:water (1:1). Percolated extract was concentrated under reduced pressure at 40 °C and lyophilized until total solvent elimination.

Total polyphenols determination

Chemical analysis was performed at samples protected from light during dilution. Water free of carbon dio-

xide was used for the experiments. Lyophilized *Syzygium cumini* extract was weighed, 0.750 g, and transferred to a 250 mL volumetric flask completed with water.

The amount of 5 mL of the above mentioned dilution was filtered and transferred to a 25 mL volumetric flask. To a 5 mL of the last solution was added 2 mL of Folin-Ciocalteu 2 N and diluted to 50 mL with sodium carbonate solution 10% w/v. Absorbance (A1) was achieved at 757.0 nm after 3 min of the addition of the last reagent. Water was considered the blank (Farmacopéia, 1988).

Analysis of polyphenols not-absorbed by skin powder

An amount of 20 mL of the lyophilized extract solution in water was filtered and added of 0.2 g skin powder. Vigorous agitation during 60 min was used to assure homogenization and, then, dispersion was filtered. 5 mL of the filtered solution was diluted in 25 mL of water. An amount of 5 mL of the last solution was added of 2 mL Folin-Ciocalteu 2 N and diluted to 50 mL with sodium carbonate solution 10% w/v. Absorbance (A2) was registered at 757.0 nm after 3 min of the addition of the last reagent. Water was considered the blank (Farmacopéia, 1988).

Reference standard stock solution

50 mg of standard pyrogalllic acid was diluted in 50 mL of water, following, 5 mL of this solution was transferred to a 100 mL volumetric flask completed with water. To 5 mL of the last solution, 2 mL of Folin-Ciocalteu 2 N were added, being then diluted to 50 mL with sodium carbonate solution 10% w/v. Absorbance (A3) was obtained at 757.0 nm, 3 min after of the addition of the last reagent and within 15 min of the standard pyrogalllic acid dilution. Water was considered the blank.

Total polyphenols were calculated according to Equation 1 (Farmacopéia, 1988):

$$TP = \frac{13.12(A_1 - A_2)}{A_3 \cdot m}$$

EQUATION 1 - Total polyphenols (TP, in g) from *Syzygium cumini* fruit extract. m: mass of the sample (g); A1: absorbance of total polyphenols from the extract; A2: absorbance of polyphenols not-absorbed by skin powder from the extract; A3: absorbance of standard.

Analytical curve and linearity

Standard pyrogalllic acid was diluted in the range of

concentrations from 0.25 to 7.5 $\mu\text{g}\cdot\text{mL}^{-1}$. The absorbance measurements were obtained at 757.0 nm using Spectrophotometer Hewlett Packard with a 1 cm quartz cuvette. Mean of eight replicates of each concentration value was employed to construct the analytical curve (Causon, 1997; Beer *et al.*, 2003; Rolim *et al.*, 2005).

The linearity was analyzed and confirmed by statistical analysis (Mulholland, Hibbert, 1997; Brasil, 2003; USP, 2003; Baby *et al.*, 2006).

Interval (range)

The interval of concentrations employed to construct the analytical curve was evaluated by determining the precision (RSD, %) and accuracy (E, %) for diluted solutions of the standard pyrogalllic acid (Fabre *et al.*, 1993; ICH Q2B, 1995; FDA, 2000; Brasil, 2003).

Estimated detection limit (LOD) and estimated quantification limit (LOQ)

Detection limit and quantification limit were estimated by the slope and mean standard deviation of concentrations employed to construct the analytical curve, according to Equation 2 and Equation 3 (Green, 1996; ICH Q2B, 1995; Jenke, 1996; USP, 2003; FDA, 2001; Brasil, 2003):

$$\text{LOD} = \frac{3.3 \sigma}{S}$$

EQUATION 2 - Detection limit. *LOD* is estimated detection limit ($\mu\text{g}\cdot\text{mL}^{-1}$); σ is mean standard deviation; *S* is slope of the analytical curve.

$$\text{LOQ} = \frac{10 \sigma}{S}$$

EQUATION 3 - Quantification limit. *LOQ* is estimated quantification limit ($\mu\text{g}\cdot\text{mL}^{-1}$); σ is mean standard deviation; *S* is slope of the analytical curve.

Precision and recovery/accuracy

Precision was evaluated as the assessment of the closeness of the results obtained in a series of measurements of a multiple sampling of the same sample, and recovery/accuracy represented the degree of match between the individual results found and a theoretical value accepted as reference (Brittain, 1998; Brasil, 2003; Shabir, 2003).

Precision and accuracy were calculated according to Equations 4 and 5, respectively:

$$\text{RSD} (\%) = \frac{\text{SD} \times 100}{C}$$

EQUATION 4 - Precision. *RSD (%)* is precision; *SD* is standard deviation; *C* is mean of calculated concentrations.

$$\text{Recovery/Accuracy} (\%) = \frac{C_{\text{spiked}} - C_{\text{extract}} \times 100}{TC}$$

EQUATION 5 - Recovery/Accuracy. C_{spiked} is total polyphenols from extract spiked with standard; C_{extract} is total polyphenols from extract; *TC* is theoretical concentration of the standard reference (pyrogalllic acid).

RESULTS AND DISCUSSION

Beyond the regulatory requirements, performance and reliability of analytical procedures are essential to the quality control of botanical raw materials. Based on the validation characteristics and necessities of guidelines presented on official and scientific literature, each analytical procedure must be validated with respect to parameters which are relevant to its performance. Parameters like linearity, range, specificity, precision, accuracy, limit of detection and limit of quantification are required to the experimental establishment of the validation process (Baby *et al.*, 2006).

The method used to quantify total polyphenols, as pyrogalllic acid equivalents, from *Syzygium cumini* (L.) Skeels fruit extract has offered linearity, over the concentration range of 0.25 to 7.5 $\mu\text{g}\cdot\text{mL}^{-1}$. Through the visual analysis of the analytical curve, the plotted data of absorbance measurements at 757.0 nm *versus* range of the concentrations generated a straight line. The spectrophotometry used in this research work presented advantages for routine utilization as: simplicity, rapidity, sensitivity, equipment convenience and relative low cost of reagents.

Besides the visual analysis of the analytical curve, the least-squares fit method was employed to achieve the linearity, as statistical tool, reported at Table I. The regression line with corresponding coefficient of linear correlation (r^2) were $y = 0.1744 \pm 0.0055)x + 0.032 (\pm 0.0034)$ and $r^2 = 0.9979 \pm 0.0010$, where: *y* is absorbance and *x* is total polyphenols concentration ($\mu\text{g}\cdot\text{mL}^{-1}$). Statistical data were exposed with their respective standard deviation \pm SD).

The results of the precision assay (RSD, %) were reported at Table II for the total polyphenols and the polyphenols not-absorbed by the skin powder for the *S. cumini* (L.) Skeels extract.

The methods demonstrated a RSD inferior than 5.0%, indicating an adequate precision for all assays (Brasil, 2003).

Recovery/accuracy data were described at Tables III and IV. It was observed a greater percentage of recovery/accuracy for the lowest concentrations of pyrogallol acid, indicating a positive correlation with that one of the extract.

The proposed method was considered accurate, as the recovery found in it totalizes the concentrations studied and showed adequate to the quantitative analysis of the extract and the standard pyrogallol acid.

After the validation of the methodology, the determination of the total polyphenols present on the extract was 27.706%.

LOD and LOQ were estimated according to the standard curve deviation and the slope of the regression line, presented at Table V. Limit values were found to be 0.21 and 0.64 $\mu\text{g}\cdot\text{mL}^{-1}$, respectively, for LOD and LOQ. Agreeing with the study interval and the accuracy obtained for the theoretical concentrations of the standard pyrogallol acid, LOQ should present a value $>0.50 \mu\text{g}\cdot\text{mL}^{-1}$, which corroborated the estimated value of $0.64 \mu\text{g}\cdot\text{mL}^{-1}$.

TABLE I - Linearity statistical data and interval study

Total polyphenols ($\mu\text{g}\cdot\text{mL}^{-1}$) ^a	A \pm SD	RSD	E
0.25	0.0515 \pm 0.000837	1.62	44.24
0.50	0.1003 \pm 0.002623	2.61	78.00
1.00	0.2074 \pm 0.003732	1.80	100.46
2.00	0.3925 \pm 0.016529	4.21	101.06
2.50	0.4836 \pm 0.023877	4.94	103.53
3.00	0.5720 \pm 0.025572	4.47	109.84
4.00	0.7456 \pm 0.028502	3.82	102.26
5.00	0.9182 \pm 0.029043	3.16	101.61
7.50	1.3101 \pm 0.036485	2.78	97.14

^aTheoretical concentrations; A \pm SD: mean absorbance \pm standard deviation (n = 8); RSD: precision, as relative standard deviation, %; E: accuracy, %.

TABLE II - Precision, as RSD%, for the total polyphenols, as pyrogallol acid equivalents, and for the polyphenols not-absorbed by the skin powder

Days of analysis	Total polyphenols, as pyrogallol acid		Polyphenols not-absorbed by the skin powder	
	Mean \pm SD	RSD%	Mean \pm SD	RSD%
1	1.011273 \pm 0.018048	1.784658	0.229662 \pm 0.006657	2.898574
2	1.022433 \pm 0.017558	1.717285	0.245913 \pm 0.007012	2.851468
3	0.985198 \pm 0.02237	2.27061	0.24612 \pm 0.007182	2.918171
Total	1.006302 \pm 0.002648	0.263156	0.240565 \pm 0.000268	0.111416

Mean \pm SD: mean of total polyphenols concentrations (n = 3) \pm Square Deviation

TABLE III - Recovery/accuracy for the total polyphenols, as pyrogallol acid equivalents, presented in the *Syzygium cumini* (L.) Skeels extract

Standard added ($\mu\text{g}\cdot\text{mL}^{-1}$)	Extract spiked	Extract sample	Pyrogallol acid concentration found	Recovery/Accuracy (%)
1.0	1.225367 \pm 0.077878	1.006302 \pm 0.002648	0.207022 \pm 0.003687	105.81725
2.0	1.422556 \pm 0.082112	1.006302 \pm 0.002648	0.391668 \pm 0.015681	106.2772
3.0	1.573678 \pm 0.090506	1.006302 \pm 0.002648	0.571703 \pm 0.02394	99.24313

TABLE IV - Recovery/accuracy for polyphenols not-absorbed by the skin powder presented in the *Syzygium cumini* (L.) Skeels extract

Standard added ($\mu\text{g}\cdot\text{mL}^{-1}$)	Extract spiked	Extract sample	Pyrogallol acid concentration found	Recovery/Accuracy (%)
1.0	0.451542 \pm 0.030494	0.229662 \pm 0.006657	0.207022 \pm 0.003687	107.17701
2.0	0.622797 \pm 0.037218	0.2459 \pm 0.007012	0.391668 \pm 0.015681	96.228693
3.0	0.82463 \pm 0.053287	0.24612 \pm 0.007182	0.571703 \pm 0.02394	101.19065

TABLE V - Estimative of limits of detection and quantification from standard curve deviation

Total polyphenols ($\mu\text{g mL}^{-1}$) ^a	SD	σ	S	LOD	LOQ
0.25	0.000837				
0.50	0.002623				
1.00	0.003731				
2.00	0.016529				
2.50	0.023877	0.011242	0.1744	0.21	0.64
3.00	0.025572				
4.00	0.028502				
5.00	0.029043				
7.50	0.036485				

^aTheoretical concentrations; SD: standard deviation; σ : mean standard deviation; S: slope from the regression line; LOD: estimated limit of detection, $\mu\text{g mL}^{-1}$ (LOD = 3.3 σ /S); LOQ: estimated limit of quantification, $\mu\text{g mL}^{-1}$ (LOQ = 10 σ /S)

CONCLUSION

A routine and economical spectrophotometric method was developed and validated for assay of total polyphenols, as pyrogallol equivalents, present in the *Syzygium cumini* (L.) Skeels extract. Method advantages were the absence of sample extraction, rapidity, direct responses and results, equipment convenience and relative low cost of reagents. The experimental results, in addition to statistical analysis, have proven that analytical parameters were precise, accurate and sensitive according to adequate linearity, recovery, repeatability and reproducibility, LOD and LOQ.

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REFERENCES

- BABY, A.R.; MACIEL, C.P.M.; KANEKO, T.M.; VELASCO, M.V.R. UV spectrophotometric determination of bioflavonoids from a semisolid pharmaceutical dosage form containing *Trichilia catigua* Adr. Juss (and) *Ptychopetalum olacoides* Bentham standardized extract: analytical method validation and statistical procedures. *J. AOAC Int.*, v.89, n.6, p.1532-1537, 2006.
- BEER, J.O.; BATEN, P.; NSENGYUMVA, C.; SMEYERS-VERBEKE, J. Measurement uncertainty from validation and duplicate analysis results in HPLC analysis of multivitamin preparations and nutrients with different galenic forms. *J. Pharm. Biomed. Anal.*, v.32, n.4/5, p.767-811, 2003.
- BRAGA, F.G.; BOUZADA, M.L.M.; FABRI, R.L.; MATOS, M.O.; MOREIRA, F.O.; SCIO, E.; COIMBRA, E.S. Antileishmanial and antifungal activity of plants used in traditional medicine in Brazil. *J. Ethnopharmacol.*, v.111, n.2, p.396-402, 2007.
- BRASIL. Resolução R.E. n. 899, de 29 de maio de 2003. Determina a publicação do Guia para validação de métodos analíticos e bioanalíticos. Available at: <<http://e-legis.bvs.br/leisref/public/showAct.php?id=15132&word>>. Access on: 26 Jan. 2004.
- BRITAIN, H.G. Validação de métodos analíticos não cromatográficos. *Pharm. Technol., Ed. Bras.*, São Paulo, v.2, n.3, p.4-9, 1998.
- CAUSON, R. Validation of chromatographic methods in biomedical analysis: view point and discussion. *J. Chromatogr. B. Biomed. Sci. Appl.*, v.689, n.1, p.175-180, 1997.
- CHANDRASEKARAN, M.; VENKATESALU, V. Antibacterial and antifungal activity of *Syzygium jambolanum* seeds. *J. Ethnopharmacol.*, v.91, n.1, p.105-108, 2004.
- FABRE, H.; SUN, S.W.; MANDROU, B.; MAILLOLS, H. Assay validation for an active ingredient in pharmaceutical formulation: practical approach using ultraviolet spectrophotometry. *Analyst Letchworth.*, v.118, n.4, p.1061-1064, 1993.
- FARMACOPÉIA Brasileira. 4.ed. São Paulo: Atheneu. 1988. pt1.
- FDA. Food and Drug Administration. Center for Drug Evaluation and Research. Chemistry (Draft). Analytical procedures and methods validation: chemistry, manufacturing and controls documentation. 2000. Available at: <http://www.fda.gov/cder/guidance/>. Access on: 12 Nov. 2005.
- FDA. Food and Drug Administration. Center for Drug Evaluation and Research. *Biopharmaceutics. Bioanalytical method validation*. 2001. Available at: <http://www.fda.gov/cder/guidance>. Access on: 12 Nov. 2005.

- GREEN, J.M. A practical guide to analytical method validation. *Anal. Chem.*, Columbus, v.68, p.305-309, 1996. Available at: <http://www.pubs.acs.org>. Access on: 12 Jun. 2004.
- GROVER, J.K.; VATS, V.; RATHI, S.S.; DAWAR, R. Traditional Indian anti-diabetic plants attenuate progression of renal damage in streptozotocin induced diabetic mice. *J. Ethnopharmacol.*, v.76, n.3, p.233-238, 2001.
- ICH Q2B. Department of Health and Human Services. Food and Drug Administration. Center for Drug Evaluation Research. *Guidance for industry: ICH Q2B Validation of analytical procedures: methodology*. Rockville, 1995. (CPMP/ICH/281/95). Available at: <http://www.fda.gov/cber/gdlns/ichq2bmeth.pdf>. Access on: 26 Jan. 2008.
- JENKE, D.R. Chromatographic method validation: a review of current practices and procedures. II. Guidelines for primary validation parameters. *J. Liq. Chromatogr. Relat. Technol.*, v.19, n.5, p.737-757, 1996.
- MIGLIATO, K.F.; BABY, A.R.; ZAGUE, V.; VELASCO, M.V.R.; CORRÊA, M.A.; SACRAMENTO, L.V.S.; SALGADO, H.R.N. Ação farmacológica de *Syzygium cumini* (L.) Skeels. *Acta Farm. Bonaer.*, v.25, n.2, p.310-304, 2006.
- MIGLIATO, K.F.; MOREIRA, R.R.D.; MELLO, J.C.P.; SACRAMENTO, L.V.S.; CORRÊA, M.A.; SALGADO, H.R.N. Controle da qualidade do fruto de *Syzygium cumini* (L.) Skeels. *Rev. Bras. Farmacogn.*, v.17, n.1, p.94-101, 2007.
- MULHOLLAND, M.; HIBBERT, D.B. Linearity and the limitations of squares calibration. *J. Chromatogr.*, v.762, n.1/2, p.73-82, 1997.
- PEPATO, M.T, MORI, D.M.; BAVIERA, J.B.; HARAMI, R.C.; VENDRAMINI, R.; BRUNETTI, I.L. Fruit of the jambolan tree (*Eugenia jambolana* Lam.) and experimental diabetes. *J. Ethnopharmacol.*, v.96, n.1-2, p.43-48, 2004.
- REYNERTSON, K.A.; YANG, H.; JIANG, B.; BASILE, M.J.; KENNELLY, M.E.J. Quantitative analysis of antiradical phenolic constituents from fourteen edible Myrtaceae fruits. *Food Chem.*, v.109, n.4, p. 883-890, 2008.
- ROLIM, A.; MACIEL, C.P.M.; KANEKO, T.M.; CONSIGLIERI, V.O.; SALGADO-SANTOS, I.M.N.; VELASCO, M.V.R. Validation assay for total flavonoids, as rutin equivalents, from *Trichilia catigua* Adr. Juss (Meliaceae) and *Ptychopetalum olacoides* Bentham (Olacaceae) commercial extract. *J. AOAC Int.*, v.88, n.4, p.1015-1019, 2005.
- SCALBERT, A. Antimicrobial properties of tannins. *Phytochem. Anal.*, v.30, n.12, p.3875-1883, 1991.
- SHABIR, G.A. Validation of high-performance liquid chromatography methods for pharmaceutical analysis. Understanding the differences and similarities between validation requirements of the US Food and Drug Administration, the US Pharmacopeia and the International Conference on Harmonization. *J. Chromatogr. A*, v.987, n.1-2, p.57-66, 2003.
- SHAFI, P.M.; ROSAMMA, M.K.; JAMIL, K.; REDDY, P.S. Antibacterial activity of *Syzygium cumini* and *Syzygium travancoricum* leaf essential oils. *Fitoterapia*, v.73, n.5, p.414-416, 2002.
- SHARMA, S.B.; NASIR, A.; PRABHU, K.M.; MURTHY, P.S.; DEV, G. Hypoglycaemic and hypolipidemic effect of ethanolic extract of seeds of *Eugenia jambolana* in alloxan-induced diabetic rabbits. *J. Ethnopharmacol.*, v.85, n.2-3, p.201-206, 2003.
- UNITED States Pharmacopeia. 27 ed. The National Formulary: NF22. Rockville: United States Pharmacopeial Convention, 2003. p.2622-2625.

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