

Validation of HPLC, DPPH[•] and nitrosation methods for mesalamine determination in pharmaceutical dosage forms

Janice Aparecida Rafael*, José Roberto Jabor, Rúbia Casagrande, Sandra Regina Georgetti, Maria de Fátima Borin, Maria José Vieira Fonseca

Departamento de Ciências Farmacêuticas, Faculdade de Ciências Farmacêuticas de Ribeirão Preto, Universidade de São Paulo

Mesalamine (5-aminosalicylic acid, 5-ASA) is used because of its local effects in the treatment of inflammatory bowel disease. Therefore, the aims of this work were to compare and validate three analytical methods for the quality control of commercial coated tablets containing 5-ASA: high performance liquid chromatography (HPLC), 1,1-diphenyl-2-picrylhydrazyl radicals (DPPH[•]) and nitrosation. The parameters linearity, precision and accuracy were studied in this work. HPLC with ultraviolet detection at 254 nm was carried out with a C18 column and a mobile phase constituted of 30 mmol/L monobasic phosphate buffer (pH 7.0) and methanol (70:30; v/v), with 25% tetrabutylammonium hydrogen sulphate. The DPPH[•] method was performed at 517 nm and using 100 mmol/L acetate buffer, pH 5.5, ethanol and 250 µmol/L ethanolic solution of DPPH[•]. The nitrosation method was accomplished by using a platinum electrode and standard 0.1 mol/L sodium nitrite as titrant solution. Repeatability (intra-day) and intermediate precision (inter-day), expressed as RSD, were lower than 3%. The experimental recoveries were between 72.5 and 99.9%. Statistical analysis by one-way ANOVA, followed by the multiple comparison test of Bonferroni showed no significant difference among the three methods. All proposed methods can be used for the reliable quantitation of 5-ASA in pharmaceutical dosage forms.

Uniterms

- Mesalamine
- High performance liquid chromatography
- 1,1-diphenyl-2-picrylhydrazyl radicals
- Nitrosation
- Validation
- Pharmaceutical dosage forms

*Correspondence:

J.A.Rafael
Faculdade de Ciências Farmacêuticas
de Ribeirão Preto
Universidade de São Paulo
Av. do Café, s/n
14040-903 - Ribeirão Preto - SP, Brazil
E-mail: jarafael@fcrp.usp.br

INTRODUCTION

Mesalamine (5-aminosalicylic acid, 5-ASA) (Figure 1) is used for its local effects in the treatment of inflammatory bowel disease, including ulcerative colitis and Crohn's disease (Cai *et al.*, 2003; Gotti *et al.*, 2001). Despite the fact that it has been used for over 50 years, the mechanism of

action of this drug remains uncertain. 5-ASA has been shown to be a potent scavenger of reactive oxygen species that play a significant role in the pathogenesis of inflammatory bowel disease, inhibition of natural killer cell activity, inhibition of antibody synthesis, inhibition of cyclo-oxygenase and lipoxygenase pathways and impairment of neutrophil function (Geier, Miner, 1992; Palumbo *et al.*, 1995).

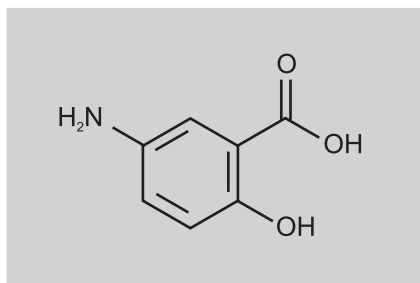


FIGURE 1 - Structure of 5-ASA.

This drug is unstable under gastric conditions and prone to be absorbed in the upper intestine, which causes low drug bioavailability and low efficiency against inflammatory colon disease. Therefore, colon-specific delivery of 5-ASA is an important issue (Cai *et al.*, 2003).

Among 5-ASA colon-specific prodrugs, the earliest accepted drug is sulfasalazine, an azo-conjugate of 5-ASA with sulfapyridine, but this drug presents adverse effects due to sulfapyridine. Four novel delivery mechanisms have been developed to delay the release of 5-ASA until it reaches the colonic site of inflammation: (1) 5-ASA conjugated to an inert molecule; (2) 5-ASA conjugated to another 5-ASA molecule; (3) 5-ASA enveloped in a delayed-release coating and (4) 5-ASA enveloped in a pH-sensitive coating (Cai *et al.*, 2003; Geier, Miner, 1992).

Polymeric prodrugs with 5-ASA can successfully deliver 5-ASA to the colon (Cai *et al.*, 2003). Asacol[®], an oral commercial formulation containing 400 mg of 5-ASA coated with acrylic resin (Eudragit S resin), delivers the drug at alkaline pH (Riley, Turnberg, 1990).

Since the drug is widely used in the treatment of inflammatory bowel disease, it is important to compare and validate analytical methods for its determination in pharmaceutical dosage forms.

Current literature reports few methods to quantify 5-ASA in pharmaceutical dosage forms. A HPLC method adopted by the United States Pharmacopoeia 24 ed. is based on the mobile phase containing tetrabutylammonium hydrogen sulphate as an ion-pairing agent, which shortens column life. Moreover, mobile phase preparation requires tedious procedures.

On the other hand, the structural characteristic of 5-ASA allows the use of other quantitative methods. Since antioxidant properties have been attributed to 5-ASA because it is a phenolic compound (Dinis, Madeira, Almeida, 1994), antioxidant activity can be used as an indirect method for 5-ASA quantitation. The DPPH[·] method is economic, simple, rapid and widely used to determine the antioxidant activity of phenolic compounds. A

nitrosation method can be used to classify amines as primary (-NH₂), secondary (-NHR), or tertiary (-NR₂). The derivative products change according to whether the amine being tested is aliphatic or aromatic, and according to its substitutions. Primary aromatic amines react with nitrous acid to form diazonium salts, which are stable at 0 °C but decompose to nitrogen gas at room temperature. Due to the formation of a diazonium salt, the method can be used as a quantitative method, applying potentiometric titration. It is a simple procedure and a reproducible method.

The present work reports the comparison and validation of the HPLC, DPPH[·] and nitrosation methods to quantify 5-ASA in coated tablets (Asalit[®] 400 mg). The work describes analytical parameters that are very important for the validation of an analytical procedure, such as accuracy and specificity.

MATERIAL AND METHODS

Chemical

A 5-ASA reference standard was obtained from Acros Organics (New Jersey, USA). The pharmaceutical dosage form (Asalit[®] 400mg) containing 5-ASA was kindly supplied by Galena (Campinas, SP, Brazil). All other reagents used were of pharmaceutical grade or HPLC grade. All solutions were prepared daily.

Experimental apparatus and analytical condition

The HPLC method was performed on a LC-10AT (Shimadzu Corporation, Tokyo, Japan) equipped with a 20 μ L loop and SPD-10A UV/VIS detector (Shimadzu). Integration of the chromatographic peaks was achieved with C-R6A Chromatopac integrator (Shimadzu). The analysis was performed by using a SuperPac Sephasil C18 column (particle size: 5 μ m; 250 x 4.0 mm i.d.; SuperPac Sephasil), and samples were isocratically eluted with a 30 mmol/L monobasic phosphate buffer (pH 7.0) and methanol (70:30; v/v), containing 25% tetrabutylammonium hydrogen sulphate, with the flow rate set at 1.0 mL/min. Each sample was filtered prior to injection using a Millex LCR filter (Millipore Corporation, São Paulo, SP, Brazil) and a 20 μ L aliquot was injected into the HPLC apparatus with UV detection at 254 nm (USP 24, 2000).

The DPPH[·] method was performed on a UV-VIS Spectrophotometer U2001 (Hitachi) at 517 nm, using 1.0 cm quartz cells. The antioxidant activity was determined by the hydrogen-donating ability of 5-ASA. For radical scavenging activity measurements, 1 mL of 100 mmol/L

acetate buffer, pH 5.5, 1 mL of ethanol, 0.5 mL of 250 µmol/L ethanolic solution of DPPH[•] was mixed, and 10 µL of different concentrations of 5-ASA were added. The absorbance was measured after 10 min at 517 nm, according to the method described by Dinis *et al* (1994). The blank was prepared from the reaction mixture without DPPH[•] solution. All measurements were performed in triplicate.

The nitrosation method was conducted according to Korolkovas (1988). The potentiometric titration procedure was carried out using a platinum electrode as indicator electrode and a Micronal B374 pHmeter was used.

0.1 mol/L sodium nitrite solution was used as titrant. Mesalamine samples was dissolved in 0.005 mol/L HCl and cooled in an ice bath. The samples were titrated with 0.1 mol/L sodium nitrite and the diazonium salts formed were determined potentiometrically.

Preparation of the standard solutions and sample solutions

Preparation of tablet samples

In order to acquire the sample solutions, 5 coated tablets were individually accurately weighted and their average weight was estimated. These 5 tablets were triturated one at a time to a powder. The remaining blended powder was used to prepare the sample solutions.

HPLC method

An amount of tablets powder corresponding to 25 mg of mesalamine was weighted and diluted to obtain a solution with 10 µg/mL final concentration in the mobile phase solution. The analytical curve was prepared with standard mesalamine in final concentrations of 0.25-100 µg/mL, originally acquired from a 1000 µg/mL mesalamine stock solution. All samples were prepared in triplicate.

1,1-diphenyl-2-picrylhydrazyl radical (DPPH[•]) method

Coated powdered samples were weighted to obtain the equivalent to 50 mg of mesalamine, and this powder was diluted with a 30 mmol/L monobasic phosphate buffer (pH 7.0) and methanol (70:30; v/v), containing 25% tetrabutylammonium hydrogen sulphate in order to obtain a 500 µg/mL. 10 µL this solution was used for the reactions in order to obtain a 2 mg/mL mesalamine solution. The analytical curve was prepared with standard mesalamine in the concentration range of 0.4 – 4 µg/mL in the reaction medium. All samples were prepared in triplicate.

Nitrosation method

Accurately weighted amounts of tablets powder equivalent to 100 mg of mesalamine were diluted with 0.005 mol/L HCl (and deionized water). The solution was cooled in an ice bath. A potentiometric titration procedure was performed by reaction of the aromatic amine (mesalamine) with the titrant solution (0.1 mol/L of sodium nitrite) in order to produce *N*-nitrosamines (diazonium salts). Low temperatures are required in the procedure to avoid diazonium salt decomposition. A platinum electrode was used as indicator electrode. A 100 mg mesalamine reference standard underwent the same sample treatment as the analyzed sample. The analyses were conducted in triplicate.

Method validation

Linearity

The analytical curve was obtained with different concentrations (10 for HPLC and 6 for DPPH[•]) of the standard solution (0.25 – 100 µg/mL for HPLC method and 0.4 – 3 µg/mL for the DPPH[•] method). The solutions were prepared in triplicate. The linearity was evaluated by linear regression analysis (Raggi *et al.*, 2000).

Since the titration methods do not need an analytical curve, linearity was not estimated for nitrosation method.

Precision

The precision of the assay was determined by repeatability (intra-day) and intermediate precision (inter-day). Repeatability was evaluated by assaying samples at same concentration during the same day. Intermediate precision was studied by comparing the assays on different days (3 days). Six sample solutions (10 µg/mL for HPLC method, 2 µg/mL for DPPH[•] method and 100 mg for nitrosation method) were prepared (Mendez, Steppe, Schapoval, 2003).

Accuracy

The accuracy was determined by recovery of known amounts of 5-ASA reference standard added to the samples at the beginning of the process. Established amounts of standard mesalamine were added to known amounts of triturated coated Asalit[®] tablets powder to obtain 3 different concentration of addition. Samples were assayed and recovery percentages for each standard concentration were estimated. The analyses were carried out in triplicate (Gotti *et al.*, 2001).

The recovery percentage of the 5-ASA standard added was calculated using the equation proposed by AOAC (1990).

RESULTS AND DISCUSSION

HPLC method

HPLC is method widely used in the quality control of drugs. Several HPLC methods have been used to measure the concentration of 5-ASA in biological fluids (Gotti *et al.*, 2001).

The HPLC method adopted by the United States Pharmacopoeia uses a mobile phase containing tetrabutylammonium hydrogen sulphate as an ion-pairing agent (USP 24, 2000). Although the column life is decreased by that compound, the addition of tetrabutylammonium hydrogen sulphate is important in order to improve the separation due to the ion-par interactions of the ammonium salts with the anionic solutes (Cendrowska *et al.*, 1990). Thus, 5-ASA present in coated tablet Asalit[®] 400 mg was determined by the HPLC method proposed by the United States Pharmacopoeia.

Figure 2 shows standard solution (A) and sample solution (B) typical chromatograms obtained from the 5-ASA analyses using the proposed method. As shown in the results, the standard and sample solutions were eluted at the same retention time (2.89 min) allowing a rapid determination of the drug, which is important for routine analysis. The chromatograms present symmetrical peaks and the base line was free of interference for both standard and sample solutions. Cendrowska *et al.* (1990) examined two HPLC methods to separate 5-ASA from other drugs, and the retention time obtained was higher than 7 min. Thus, the proposed methodology is faster than that described by Cendrowska *et al.* (1990).

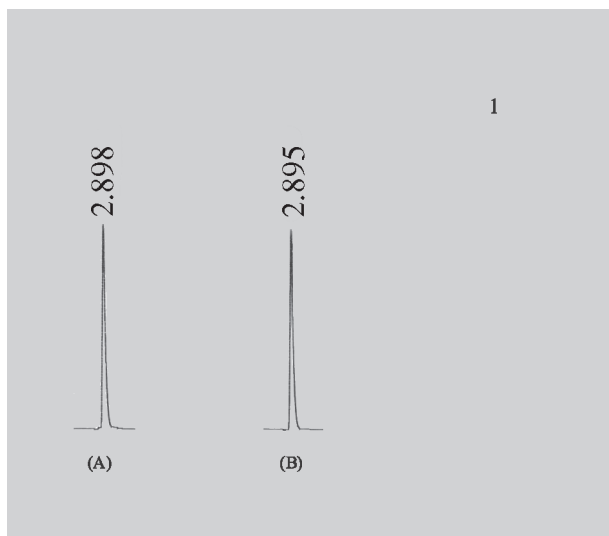


FIGURE 2 - HPLC chromatograms of 5-ASA reference standard (A) and 5-ASA present in coated tablet Asalit[®] 40 µg/mL (B).

The analytical curve for 5-ASA was constructed by plotting drug concentration versus peak height and showed good linearity in the 0.25–100 µg/mL range. The representative linear equation was $y = 437.41x + 60.915$, with a correlation coefficient ($r = 0.999$) highly significant for the method (Table I).

Method precision was determined by repeatability (intra-day) and inter-day intermediate precision (inter-day) tests and was expressed as RSD (relative standard deviation) (%) of a measurement series. The analytical parameters obtained for the determination of 5-ASA by the HPLC method are presented in Table II. The experimental values obtained over 3 days for a measurement series of a 10 µg/mL solution showed 1.75% RSD, indicating good inter-day precision. Intra-day variability showed a mean RSD of 2.51%. Haney and Dash (1997) validated the HPLC method for 5-ASA assay and the intra and inter-day precisions obtained were 1.2–4.6% and 1.1–3.5%, respectively.

The USP and AOAC indicate, in general, RSD values lower 2% (AOAC, 1975; Parejo *et al.*, 2000). According to Raggi *et al.* (2000) the RSD value should be lower than 3%. Therefore, the precision of this method is within the expected range.

The accuracy of the method was determined by addition of three different concentrations of standard in the samples, in triplicate, and the mean recovery percentage was found to be 88.2% (Table III). In general, the recovery percentage as a measure of the accuracy should be close to 100% (AOAC, 1975; Parejo *et al.*, 2000).

1,1-diphenyl-2-picrylhydrazyl radicals (DPPH[•]) method

DPPH[•] is a stable free radical potentially reactive with substances able to donate a hydrogen atom and, thus, useful to assess antioxidant activity of specific compounds of extracts (Dinis, Madeira, Almeida, 1994). Because of its odd electron, DPPH[•] has a strong absorption band at 517 nm. Since this electron becomes paired in the presence of a free radical scavenger, the absorption decreases stoichiometrically with respect to the number of electrons taken up. This change in absorbance produced by this reaction has been widely used to test the ability of several molecules to act as free radical scavengers (Dinis, Madeira, Almeida, 1994).

Various investigations have shown that 5-ASA presents antioxidant activity (Dinis, Madeira, Almeida, 1994) demonstrated the antioxidant activity of 5-ASA compared to other antipyretic and anti-inflammatory drugs, and indicated that 5-ASA acts as a potent free radical scavenger, in a way similar to ascorbate, in contrast with salicylate which does not react with DPPH[•]. Among the compounds tested, 5-ASA presented the highest antioxidant activity (Dinis, Madeira, Almeida, 1994).

TABLE I - Regression analysis of the quantitation data of 5-ASA by two of the proposed methods

Statistical parameters	HPLC Method	DPPH [•] Method
Regression equation ^a	$y=437.41x + 60.915$	$y=10.707x - 0.6435$
Standard error of slope	102.78	1.01
Standard error of intercept	1.88	0.00218
Correlation coefficient (r)	0.999	0.999
Concentration range (µg/mL)	0.25 – 100	0.4 - 3

$y=ax + b$, x is the concentration of the drug in µg/mL (HPLC method and DPPH[•] method) and y is peak high (HPLC method) or antioxidant activity (DPPH[•] method); ^a Based on three calibration curves.

TABLE II - Analytical parameters obtained in validation of the proposed methods

	HPLC (10 µg/mL)	DPPH [•] (2 µg/mL)	Nitrosation (100 mg)
mean	9.55 µg/mL	2.31 µg/mL	97.64 mg
SD	1.68	3.02	1.07
RSD (%) Intra-day	2.51	1.50	1.93
RSD(%) Inter-day ^a	1.75	2.60	1.09

SD = standard derivation, RSD = relative standard deviation in percentage; ^a3 days

TABLE III - Experimental values obtained in the recovery test for 5-ASA in commercially coated tablet

Method	Sample concentration	Concentration of added standard	% Recovery ^a ± RSD (%)
*HPLC	30 µg/mL	10 µg/mL	98.0 ± 4.63
	30 µg/mL	20 µg/mL	75.5 ± 1.80
	30 µg/mL	30 µg/mL	91.0 ± 2.00
*DPPH	200 µg/mL	200 µg/mL	72.5 ± 2.00
	200 µg/mL	300 µg/mL	82.5 ± 2.30
	200 µg/mL	400 µg/mL	81.4 ± 1.38
Nitrosation	50 mg	30 mg	99.9 ± 1.60
	50 mg	40 mg	97.9 ± 1.28
	50 mg	50 mg	95.9 ± 1.80

^a Mean of three determination; * Aliquot of 20 mL and 10 mL of the solutions were used for the HPLC and DPPH methods, respectively.

In the present study, the antioxidant activity was determined by the ability of 5-ASA to donate hydrogen atoms to DPPH[•]. It was utilized in order to quantify 5-ASA in Asacol[®] 400 mg coated tablets, using spectrophotometric methods, and this was compared to other methodologies proposed to quantify 5-ASA.

5-ASA caused an instantaneous decrease in DPPH[•] absorbance, when compared to the control absorbance, suggesting that the commercial coated tablets present high antioxidant activity.

A concentration-response curve was constructed by plotting 5-ASA concentration versus free radical scavenger activity and linearity was obeyed for the range of 0.4-3 µg/mL in the reaction medium. The representative linear equation was $y = 10.707x - 0.6435$, with a correlation coefficient of 0.999 (Table I).

Table II shows the analytical parameters obtained for the determination of 5-ASA by the DPPH[•] method, using a 2 µg/mL solution in order to acquire the measurement series. The results indicate satisfactory intra-day variability

(1.50% RSD) and inter-day variability (2.60% RSD). The accuracy of the method was determined. However, the mean recovery percentage was 78.8% (Table III), which is under the recommended recovery percentage value of 100%.

Nitrosation method

Amine nitrosation is a quantitative analytical method that uses a standard sodium nitrite solution as titrant. The titration is carried out in an acidic environment. Nitrous acid reacts quantitatively with aromatic amines producing diazonium salts. Since it is a simple and reproducible method, it can be used to quantify 5-ASA, which is an aromatic amine, present in commercial coated tablets.

Experimental data obtained when 5-ASA was quantified through the nitrosation method were used to set the analytical parameters shown in Table II. The results show a 1.11% RSD, pointing to a satisfactory intra-day variability, and a 1.09% RSD inter-day variability. The accuracy of the method was estimated and the mean recovery percentage was found to be 97.2%.

Comparison between proposed methods

The proposed analytical methods were compared using statistical analysis. Data were statistically analyzed by one-way ANOVA, followed by the Bonferroni multiple comparison test and do not show significant differences among the methods, considering $P > 0.05$.

HPLC and DPPH[•] methods had very similar precisions, both inside the acceptable limit. HPLC had a better recovery percentage than DPPH[•], which showed recoveries under the limit suggested by AOAC (1975).

CONCLUSIONS

HPLC, DPPH[•] and nitrosation are all suitable methods for a reliable quantitation of 5-ASA in commercial coated tablets in terms of analytical parameters. There were no significant statistical differences among the proposed methods. It should be noted that all methods showed similar and favorable results with respect to precision and accuracy. The RSD values are all lower than 3%. However, the nitrosation method showed the best recovery percentage and it was the most precise method, as well as being the simplest and most inexpensive methodology. The proposed methods have the advantage of using feasible analytical procedures and need only a simple preparation of the samples. Therefore, they can be used for the drug analysis in routine quality control.

RESUMO

Validação dos métodos de CLAE, DPPH[•] e nitrosação para determinação de mesalazina em formas farmacêuticas

Mesalazina (ácido 5-aminosalicílico, 5-ASA) é utilizado devido seu efeito local no tratamento de doença inflamatória intestinal. Assim, o objetivo deste trabalho foi comparar e validar três métodos analíticos para o controle de qualidade de comprimidos comerciais revestidos contendo 5-ASA: cromatografia líquida de alta eficiência (CLAE), radical 1,1-difenil-2-picril-hidrazil (DPPH[•]) e nitrosação. Os parâmetros linearidade, precisão e exatidão foram estudados neste trabalho. CLAE com detecção ultravioleta em 254 nm foi realizada utilizando coluna C18 e a eluição em fase móvel constituída de tampão fosfato monobásico 30 mmol/L (pH 7,0) e metanol (70:30; v/v), com 25% de sulfato hidrogênio de tetrabutilamônio. Para o método de DPPH[•] utilizou-se tampão acetato 100 mmol/L, pH 5,5, álcool etílico e 250 µmol/L solução etanólica de DPPH[•] a 517 nm. Para o método de nitrosação utilizou-se um eletrodo de platina e um padrão de nitrito de sódio 0.1 mol/L como solução titulante. Repetibilidade (intra-dia) e precisão intermediária (inter-dia), expressado como DPR, foi menor que 3%. A recuperação experimental foi entre 72,5 e 99,9%. Análise estatística por "one-way" ANOVA, seguida de comparação múltipla do teste de Bonferroni, não mostrou significância entre os três métodos. Os métodos propostos podem ser usados para análise quantitativa de 5-ASA em formas farmacêuticas.

UNITERMOS: Mesalazina. Cromatografia líquida de alta eficiência. 1,1-difenil-2-picril-hidrazil. Nitrosação. Validação. Forma farmacêutica.

ACKNOWLEDGMENTS

This work was supported by Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES). Janice A. Rafael received a fellowship from CNPq. The authors would like to thank Galena (Campinas, SP, Brazil) for supplying 5-ASA.

REFERENCES

ASSOCIATION OF OFFICIAL ANALYTICAL CHEMISTS. Official Methods of Analysis. 15. ed. Arlington: Association of Official Analytical Chemists, 1990. v.1, p. 17.

- ASSOCIATION OF OFFICIAL ANALYTICAL CHEMISTS. Statistical Manual of the AOAC. 12 ed. Washington: AOAC, 1975. p. 1094.
- CAI, Q. X.; ZHU, K. J.; CHEN, D.; GAO, L. P. Synthesis, characterization and in vitro release of aminosalicic acid and 5-acetyl aminosalicic acid of polyanhydride – P (CBFAS). *Eur. J. Pharm. Biopharm.*, Amsterdam, v. 55, p. 203-208, 2003.
- CENDROWSKA, I.; DREWNOSKA, A.; GRZESZKIEWICZ, A.; BUTKIEWICZ, K. Investigation of the stability of 5-aminosalicylic acid in tablets and suppositories by high-performance liquid chromatography. *J. Chromatogr.*, Amsterdam, v. 509, p. 195-199, 1990.
- DINIS, T.C.P.; MADEIRA, V.M.C.; ALMEIDA, L.M. Action of phenolic derivatives (acetaminophen, salicylate and 5-aminosalicylate) as inhibitors membrane lipid peroxidation and peroxy radical scavengers. *Arch. Biochem. Biophys.*, New York, v. 315, n.1, p. 161-169, 1994.
- GEIER, D.L.; MINER, P.B. New therapeutic agents in the treatment of inflammatory bowel disease. *Am. J. Med.*, New York, v.93, n.2, p.208-298, 1992.
- GOTTI, R.; POMPONIO R.; BERTUCCI, C.; CAVRINI, V. Determination of 5-aminosalicylic acid related impurities by micellar electrokinetic chromatography with an ion-pair reagent. *J. Chromatogr. A*, Amsterdam, v. 916, p. 175-183, 2001.
- HANAUER, S.B. Inflammatory bowel disease revisited: Newer drugs. *Scand. J. Gastroenterol.*, Oslo, v. 25, n. 175, p. 97-106, 1990.
- HANEY, P.W.; DASH, A.K. Simple liquid chromatographic method for the analysis of 5-aminosalicylic acid and its degradation product. *J. Chromatogr. A*, Amsterdam, v.765, p. 233-238, 1997.
- KOROLKOVAS, A. *Análise farmacêutica*. Rio de Janeiro: Guanabara Koogan, 1988. p.159.
- MENDEZ, A.S.L.; STEPPE, M.; SCHAPOVAL, E.E.S., Validation of HPLC and UV spectrophotometric methods for the determination of meropenem in pharmaceutical dosage form. *J. Pharm. Biomed. Anal.*, Oxford, v.33, p.947-954, 2003.
- PALUMBO, G.; CARLUCCI, G.; MAZZEO, P. *et al.*, Simultaneous determination of 5-aminosalicylic acid, acetyl-5-aminosalicylic acid and 2, 5-dihydroxybenzoic acid in endoscopic intestinal biopsy samples in humans by high-performance liquid chromatography with electrochemical detection. *J. Pharm. Biomed. Anal.*, Oxford, v.14, p.175-180, 1995.
- PAREJO, I.; CODINA, C.; PETRAKIS, C.; KEFALAS, P. Evaluation of scavenging activity assessed by Co (II)/EDTA-induced luminol chemiluminescence and DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical assay. *J. Pharmacol. Toxicol. Methods*, New York, v.44, p.507-512, 2000.
- RAGGI, M.A.; CASAMENTI, G.; MANDRIOLI, R.; IZZO, G.; KENNDLER, E. Quantitation of olanzapine in tablets by HPLC, CZE, derivative spectrometry and linear voltammetry. *J. Pharm. Biomed. Anal.*, Oxford, v.23, p. 973-981, 2000.
- RILEY, S.A.; TURNBERG, L.A. Sulphasalazine and the aminosalicylates in the treatment of inflammatory bowel disease. *Q. J. Med.*, Oxford, v.75, n.278, p. 551-562, 1990.
- UNITED States Pharmacopeia 24. ed. Rockville, United States Pharmacopeial Convention, 2000. [CD-ROOM].

Recebido para publicação em 17 de agosto de 2005.

Aceito para publicação em 01 de dezembro de 2006.