Validation of Ultraviolet-visible and High-performance Liquid Chromatographic Methods for the Determination of Sodium p-Aminosalicylate and m-Aminophenol in a New Pharmaceutical Formulation

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ABSTRACT

Sodium p-aminosalycilate is an orphan drug used in patients affected with Multidrug-resistant Tuberculosis. Two methods, high-performance liquid chromatographic and ultraviolet spectrophotometric for the quantitative determination of sodium p-aminosalycilate and its degradation product m-aminophenol in a new pharmaceutical formulation, powder for extemporaneous reconstitution, were developed in the present work. The parameters linearity, precision, accuracy, specificity, robustness, limit of detection, and limit of quantification were also studied. Chromatography was carried out by reverse-phase technique on an RP-18 column with a mobile phase composed of 50 mM monobasic/dibasic phosphate buffer and methanol (42.5:42.5:15 v/v/v) with 1.9 g of hidroxytetrabutylammonium ionic pare adjusted to pH 7.0 with orthophosphoric acid. The ultraviolet spectrophotometric method was performed at 254 nm and 280 nm for quantification of sodium p-aminosalycilate and m-aminophenol, respectively. The proposed methods are highly sensitive, precise, and accurate and can be used for the reliable quantification of sodium p-aminosalycilate in the new alternative formulation. High-performance liquid chromatographic approach demonstrated to be a stability-indicating method, therefore suitable for the investigation of the chemical stability of sodium p-aminosalycilate.

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INTRODUCTION

Tuberculosis (TB), a pervasive and deadly infectious disease of the respiratory system, is one of the main challenges in public health and the leading cause of death among people living with the acquired human immunodeficiency syndrome. According to the World Health Organization, one-third of the world's population is infected with Mycobacterium tuberculosis.[1-3] The greatest threat to global TB control is the growing prevalence of highly resistant strains, causing Multidrug-resistant TB (MDR-TB) and extensive drug resistance.[4,5] In these cases, patients are treated with second-line atituberculosis agents such as para-aminosalicylic acid (PAS) or its sodium salts PASS (Figure 1).[6,7] They could be formulated as tablets, powder, or granules, with a suggested daily dosage of 10 g to 12 g.[6-8]

At present, granules and tablets of PAS could be available in the market, but an erratic reliable supply is frequent in less-developed countries. In addition, the high doses and bad taste of PAS and PASS make adherence compliance to a treatment very problematic. Powder dosage forms are mentioned in literature and also by physicians, but are not available although could be a convenient alternative for MDR-TB treatment.[8-10] The principal limitation of this type of pharmaceutical formulation is the moisture and heat sensitivity of PAS and PASS, since they decarboxilate to yield m-aminophenol (MAP), as a primary degradation product (Figure 1).[7,8] The maximum rate of decarboxilation is near to isoelectric pH (pI=2.71), and the minimum rate is

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between pH 9.2 and 9.5.[8] The MAP is a toxic compound, so it is very important to keep its concentration below 0.25% w/w in PASS or PAS pure drug substance and 1% w/w in PAS or PASS tablets.[10]

The authors have designed and developed a new simple powder pharmaceutical formulation for oral administration in solution after extemporaneous reconstitution (PASS-EXT).[8] In order to promote accessibility to MDR-TB treatment, the formulation is low cost and simple to manufacture. To improve patient compliance and treatment adherence, the unpleasant taste of PASS was masked, and the solution is also easy to drink. In addition, PASS-EXT remained stable when stored for three months in light resistant, glass containers, as analytical and flowability studies demonstrated.[8]

The need to take further steps to address formulation development, analytical methods should be developed and validated for assaying PASS and MAP in PASS-EXT and also for long-term stability studies.

Literature surveys revealed many reported procedures for similar purposes.[11-15] Colorimetric[11] and spectrophotometric determinations of PAS in the presence of its degradation product MAP[12,13] were informed. Quantitative analyses of them in different pharmaceutical formulations were also described by Spell et al[14] and Vasbinder et al.[15] Furthermore, in several pharmacopoeias,[10] official monographs of PASS.2H_2O, PAS, MAP in bulk drug, and PAS tablets are also included. However, as far as we know, there are no reported specific analytical methods to determine the content of PASS and MAP in a pharmaceutical formulation as the one developed in our laboratories.[8]

The present work describes the development and validation of a reversed-phase high-performance liquid chromatographic (RP-HPLC) and ultraviolet (UV)-spectrophotometric methods for the assay of PASS in bulk and in PASS-EXT. In fact, both methods were validated in relation to specificity, linearity, precision, accuracy, robustness, limit of detection (LOD), and quantitation (LOQ) according to International Council for Harmonisation (ICH) guidelines.[16-18] Besides, the RP-HPLC demonstrated to be a simple, accurate, precise, and reliable stability-indicating procedure for the simultaneous determination of PASS and MAP in the presence of the excipients of the pharmaceutical formulation. Although the UV method is not appropriate for this purpose, it is simple, rapid, economical, and the determination of PASS with proper reliability for other assays at elaboration phases is allowed.

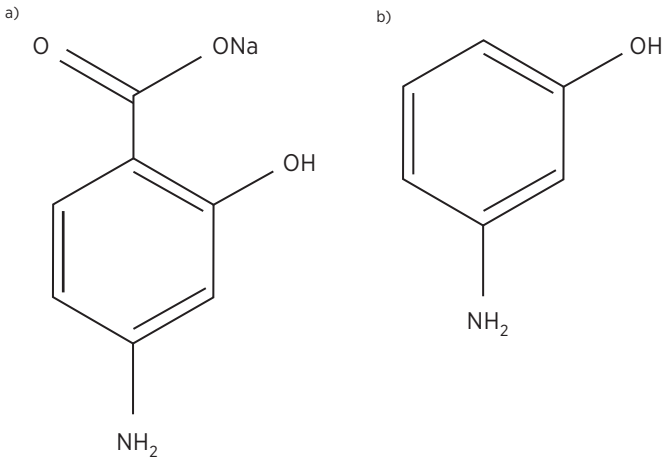
EXPERIMENTAL

MATERIALS AND INSTRUMENTATION

PASS as the dehydrate (PASS.2H_2O [99.98% purity]) was kindly supplied by Libertad Pharmaceuticals & Co. (Lot B01072; Capital Federal, Buenos Aires, Argentina). Colloidal silicon dioxide (Aerosil 200) was used as received from Degussa AG, Dusseldorf, Germany, and vanillin (Lot 010589; Saporiti Pharmaceuticals & Co., Capital Federal, Buenos Aires, Argentina) and aspartame (Lot 010423; Saporiti Pharmaceuticals & Co.) were used without further purification.

The pharmaceutical formulation was prepared in our laboratory as previously reported.[8] To use as references, PASS.2H_2O (Lot 9965E; MP Biomedicals, Inc., Illkirch, France) and MAP (Lot S14401-194; Aldrich Chemical Co., Steinheim, Germany) were used as references, respectively. Sulfanilamide (Lot 296022) and acetaminophen (Lot 186025) chemical reference standards were provided by Pharmacology and Bromatology National Institute, ANMAT, Buenos Aires, Argentina.

FIGURE 1. Chemical structures of: a) p-aminosalicylate sodium; b) m-aminophenol.



Sodium dihydrogenphosphate (Lot 5789) and sodium hydrogenphosphate (Lot 4279) were provided by Mallinckrodt Chemicals Works, St. Louis, Missouri. Methanol (Lot B37E73; J. T. Baker, Phillipsburg, New Jersey) HPLC-grade and Milli-Q water were also used. Phosphoric acid was obtained from Merck & Co., Darmstadt, Germany, and was used as a pH modifier. All solutions were prepared daily.

RP-HPLC determinations were carried out on a Spectra SYSTEM P2000 system, operating at 25°C±1°C, equipped with a model LC-10AD pump, UV/Visible (Vis) detector Thermo Separation Product, Rheodyne injector model 7125 fitted with 20 µL loop and an integrator Chromatopac model (Spectra SYSTEM).

The UV spectrophotometric studies were carried out on Shimadzu Model UV-160A spectrophotometer at λ 254 nm and 280 nm by using 1.0 cm quartz cells. The pH values were measured by an Orion SA 520 pH meter.

CHROMATOGRAPHIC CONDITIONS

The RP-HPLC analytical conditions as described in *United States Pharmacopeia (USP)* Chapter 24 for the assay of PASS.2H_2O and MAP in tablets were adapted.[10] The reverse-phase technique used a Merck Hypersil ODS-C18 column (150 mm/4.0 mm i.d. 5 µm particle size). An extensive series of preliminary assays with different compositions of mobile phases and flow rates were performed to deter-

mine the optimum analytical conditions. The samples were eluted isocratically with a flow rate of 1.0 mL/min by using a mobile phase consisting of 50 mM monobasic:dibasic phosphate buffer and methanol (42.5:42.5:15 v/v/v) with 1.9 g of hidroxytetrabutylammonium ionic pare. The pH was adjusted to 7.0 with orthophosphoric acid. The wavelength of the UV/Vis detector was set to λ 254 nm and 280 nm for quantification of PASS.2H_2O and MAP, respectively. The mobile phase was prepared daily, filtered through a 0.45-mm membrane filter (Millipore) and sonicated before use. Acetaminophen and sulfanilamide were used as internal standard for assaying PASS.2H_2O and MAP, respectively. The RP-HPLC system was operated at 23°C ±1°C.

PREPARATION OF STANDARD SOLUTIONS

About 500 mg of accurately weighed acetaminophen internal reference standard (ISA) were dissolved in the mobile phase to make 100-mL solution (final concentration 5 mg/mL). Standard solution of PASS.2H_2O was prepared by accurately weighing about 69 mg of PASS.2H_2O reference standard (SSP) and dissolving it in 50-mL mobile phase; then, 10 mL of ISA solution was added to get a final volume of 100 mL with mobile phase.

For the standard solution preparation of sulfanilamide, about 5 mg of accurately weighed internal reference standard (ISS) was transferred to a 10-mL volumetric flask and dissolved in mobile phase. A 1-mL aliquot of the resulting solution was further diluted to 100 mL with mobile phase (final concentration 5 µg/mL). Finally, 10 mL of this solution was mixed and diluted with mobile phase to obtain a volume of 100 mL (final concentration 0.5 µg/mL). Standard solution of MAP was prepared by transferring about 12 mg of accurately weighed MAP reference standard (SSM) to a 10-mL volumetric flask and volume was made up with mobile phase. A 1-mL aliquot of the resulting solution was further diluted to 100 mL with the same diluent to get a concentration 12 µg/mL. Finally, 10 mL of this solution and 10 mL of ISS were mixed and diluted with mobile phase to obtain a volume of 100 mL (final concentration of 1.2 µg/mL).

PREPARATION OF SAMPLE SOLUTIONS

The sample solution for PASS.2H_2O determination was made by transferring an accurate volume of powder formulation after reconstitution, equivalent to about 69 mg of PASS.2H_2O into a 100-mL volumetric flask. It was dissolved with 50 mL of mobile phase and mixed; then 10.0 mL of ISA solution was added and diluted with mobile phase to volume, and vortered for about 5 minutes.

The sample solution for MAP determination was made by transferring an accurate volume of powder formulation after reconstitution, equivalent to about 69 mg of PASS.2H_2O into a 100-mL volumetric flask. It was dissolved with 50 mL of mobile phase and mixed; then 10.0 mL of ISA solution was added and diluted with mobile phase to a final volume, and shaked for about 5 minutes.

ULTRAVIOLET METHOD

PREPARATION OF STANDARD SOLUTIONS

Standard solution of PASS.2H_2O was prepared by accurately weighing about 29 mg of PASS.2H_2O reference standard (SSP) and dissolving it in 250 mL of ethanol (final concentration 0.012 mg/mL). Aliquots of this solution were diluted with the same solvent at concentrations in the range of 6.37 to 9.88 µg/mL.

PREPARATION OF SAMPLE SOLUTIONS

An accurately weighed amount of powder for reconstitution equivalent to 2.9 mg ± 1 mg of PASS.2H_2O was transferred to a 250-mL volumetric flask and dissolved in ethanol (final concentration of 0.012 mg/mL). Aliquots of this solution were diluted with the same solvent at concentrations in the range of 5.88 to 10.92 µg/mL.

METHOD VALIDATION

The HPLC and UV methods were validated according to ICH guidelines for validation of analytical procedures.[16-18] The following parameters were addressed:

- Linearity
- Precision
- Accuracy
- Specificity
- Robustness
- LOD
- LOQ
- Stability of PASS.2H_2O in mobile phase

LINEARITY

The calibration curves of the standard solutions of SSP and SSM were obtained at seven different concentrations equally distributed in the range from 483 to 897 µg/mL for PASS.2H_2O and 0.7 to 9.88 µg/mL for MAP in the RP-HPLC method, and 5.88 to 10.92 µg/mL for MAP in the UV method. The solutions were prepared in triplicate. The linearity was evaluated by linear regression analysis, which was calculated by the least square regression 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 concentrations, during the same day. The intermediate precision was studied by comparing the assays on different days (3 days). The precision data were obtained by analyzing one concentration, 690 µg/mL of PASS.2H_2O and 1.2 µg/mL of MAP for RP-HPLC method and 8.4 µg/mL of PASS.2H_2O for UV method, being the precision calculated as the relative standard deviation (RSD).

ACCURACY

The accuracy of the methods was determined recovering of known amounts of SSP and ISA reference standards added to the samples at the beginning of the process.

For the RP-HPLC method, an aliquot of the fresh reconstituted powder equivalent to 0.75 g of PASS.2H_2O was transferred to a 100-mL volumetric flask which contained 10 mL of SPA, and it was dissolved in ultrapure water. Aliquots of this solution were transferred into 5-mL volumetric flasks to give final concentrations of 552, 690, and 828 µg/mL of PASS.2H_2O, and 1.4, 4.24, and 8.47 µg/mL of MAP.

For the UV method, an aliquot of the fresh reconstituted powder equivalent to 2.9 mg of PASS was transferred to a 250-mL volumetric flask and dissolved in ethanol (final concentration of 0.012 mg/mL). Aliquots of 5.0 mL of this stock solution were transferred into 10-mL volumetric flasks containing 1.0, 2.0, and 3.0 mL of SSP solution and ethanol was added to reach a final volume in order to give a final concentration of 6.0, 8.4, and 9.6 µg/mL of PASS.2H_2O, and 1.4, 4.24, and 8.47 µg/mL of MAP.

All solutions were prepared in triplicate and assayed. The percentage recovery of added PASS.2H_2O and MAP standard was calculated by using the modificated equation proposed by the *USP* monograph.[10]

SPECIFICITY

For RP-HPLC method, the specificity of the analytical method was demonstrated by extracting and analyzing spiked PASS.2H_2O, ISA, ISS, and SSM.

Identification of PASS.2H_2O, ISP, and ISM, and its degradation product MAP, was performed by RP-HPLC, and their retention times revealed no interference of the analytes with the corresponding matrix by the observation of an adequate separation among all compounds.

ROBUSTNESS

The robustness of the RP-HPLC method was determined analyzing samples under a variety of conditions such as changing equipment, mobile phases (ionic pair), and the operator. The effect on retention time and percent recovery was studied.

LIMIT OF DETECTION AND LIMIT OF QUANTIFICATION

The samples were spiked with decreasing analyte concentrations before being analyzed. The LOD was determined as the lowest amount of analyte in each sample which could be detected with a between-run coefficient of variation (RSD) <20%, but not necessarily could be quantified as an exact value. In addition, the LOQ was calculated as the lowest amount of analyte in a sample which could be quantitatively determined with an adequate precision and accuracy.[19] The parameters LOD and LOQ were determined on the basis of response and slope of the regression equation. The results were the means value of five determinations.

STABILITY OF SOLUTION OF SSP

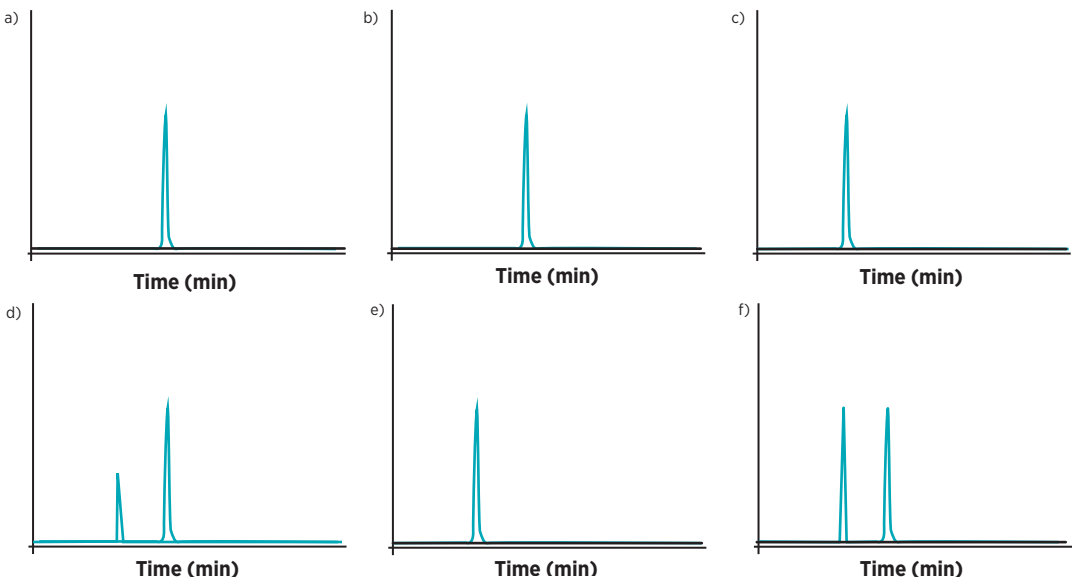
The stability of PASS.2H_2O standard reference in mobile phase preparation was investigated by storing solutions of SSP (690 µg/mL) at 5°C and 25°C for 24 hours. RP-HPLC was selected as analytical technique and samples were prepared and tested in triplicate. Chromatographic patterns were compared with that of freshly made solutions.

RESULTS AND DISCUSSION

HIGH-PERFORMANCE LIQUID CHROMATOGRAPHIC METHOD

The development of the HPLC analysis for the assay of drugs is one of the most accepted methods at present because of its versatility and reliability in routine, quality pharmaceutical analysis. A RP-HPLC method was proposed for the estimation of PASS and MAP in the pharmaceutical dosage form, and that described in *USP* Chapter 24 was taken as reference to develop and validate the procedure reported here.[10] The chromatographic conditions were adjusted in order to provide a good performance of the assay. Mobile phase selection (see the section titled Chromatographic Conditions) was based

FIGURE 2. RP-HPLC chromatograms used for quantification of PASS (UV, λ=254 nm), and MAP (UV, λ=280 nm) in PASS-EXT. a) PASS standard reference (690 µg/mL), b) PASS in the formulation (690 mg/mL), c) MAP standard reference (1.2 µg/mL), d) MAP in the formulation, e) Sulfanilamide (500 µg/mL), f) Acetaminophen in PASS-EXT (500 µg/mL).



on peak parameters (symmetry, tailing), run time, ease of preparation, stability of analytes, and cost.

Preliminary HPLC assays have been carried out to investigate the possibility of determining PASS.2H_2O in the presence of MAP (principal degradation product) and excipients (vanillin, aspartame, colloidal silicon dioxide) in the new pharmaceutical formulation. At first, PASS.2H_2O and MAP, dissolved in the mobile phase, were measured separately; sulfanilamide and acetaminophen were used as internal standard, respectively. Therefore, the good selectivity demonstrated by the proposed methodology allows selecting it in routine analysis. Figure 2 shows typical chromatograms obtained using authentic samples under the described analytical conditions. The chromatograms show the separation of the degradation product and internal standards and the drug peak with an excellent selectivity for PASS (time retention 4.26 minutes) and MAP in the formulation (time retention 3.00 minutes).

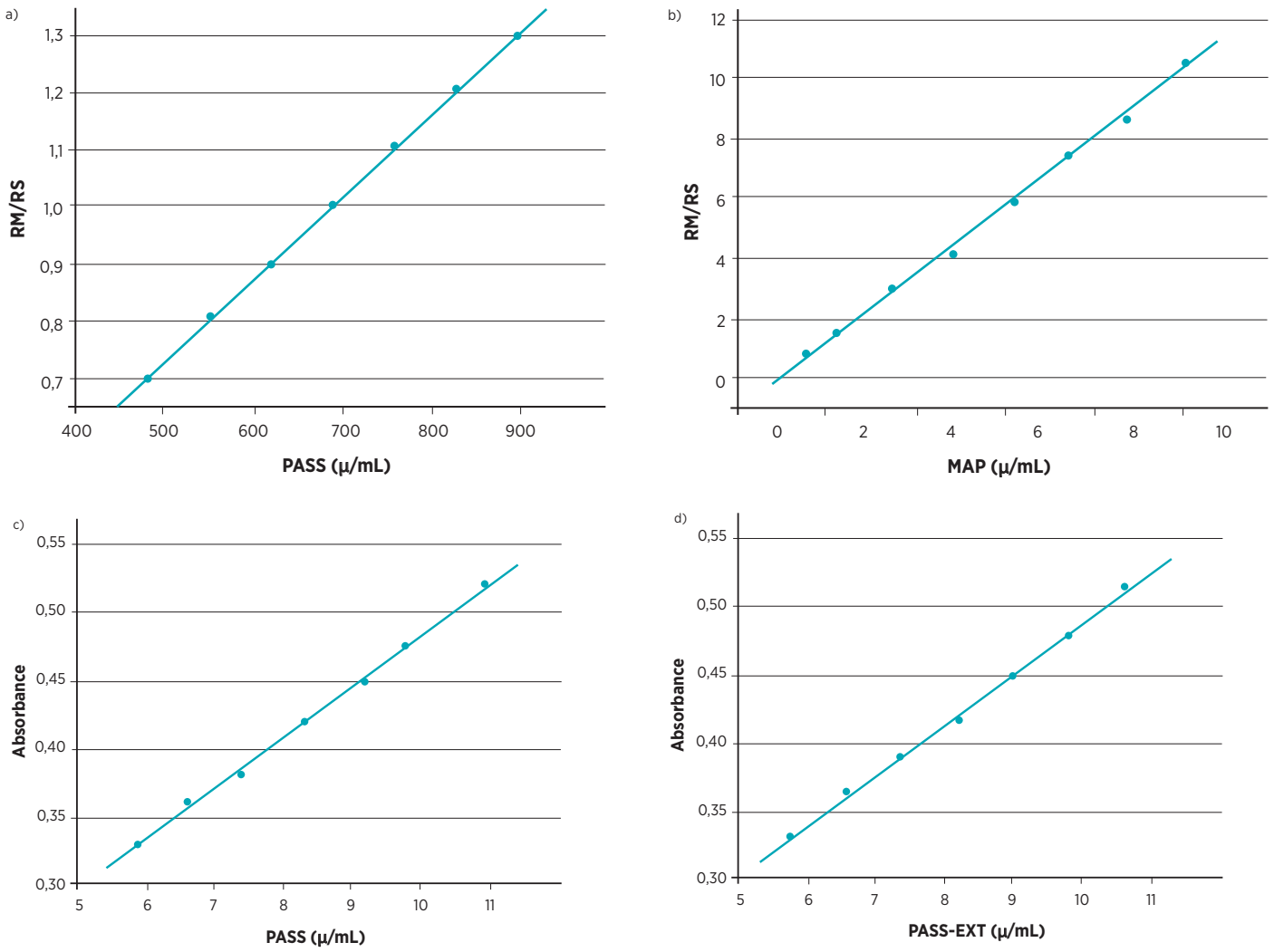
The calibration curves showed good linearity in the range 483 to 897 µg/mL for PASS.2H_2O and 0.7 to 9.88 µg/mL for MAP. The regression equations derived from the least-square method were:

$y_1 = 0.00145x_1 + 0.0386$ for PASS
and
$y_2 = 1.044x_2 - 0.0345$ for MAP

with highly significant correlation coefficients (r = 0.999) for both PASS.2H_2O and MAP (Figure 3, Table 1).

FIGURE 3. Calibration curves of a) PASS and b) MAP by HPLC method; c) PASS and d) PASS-EXT by UV method.

HPLC = high-performance liquid chromatographic; RM = reference material; RS = reference standard; UV = ultraviolet



The LOD and LOQ were found to be 0.016 and 0.050 µg/mL for PASS.2H_2O and 0.045 and 0.13 µg/mL for MAP, respectively. These results supported the high sensitivity of the RP-HPLC method developed, which could be applied for both assays, the active pharmaceutical ingredient, and its limiting impurity.

The precision of the method was determined by repeatability (intra-day) and intermediate precision (inter-day) and was expressed as RSD (%) of a series of measurement, and its results corresponded to the average of six injections. Table 2 describes experimental values obtained for the determination of PASS and MAP in samples. The calculated RSD of 0.47% for MAP and 0.79% for PASS confirmed the good intra-day precision. Inter-day variability was determined by replicate analysis of the solutions on 3 different days and precision, expressed as RSD, showed a result of 0.95% for MAP and 1.23% for PASS. Therefore, intra-day and inter-day precision was also within acceptability criteria.

The accuracy of the method was determined and the mean recovery was found to be 100.21% for PASS and 100.17% for MAP (Table 3) indicating an agreement between the true and measured values.

The described HPLC is specific. No interfering peaks were observed in degradated solutions, and the degradation product was observed at relative retention time of 3.00 minutes (Figure 4). The method was found to be robust when the mobile phase (ionic pair), operator, and instrumental were varied. During these determinations, the retention times, area and symmetry of peaks were conserved (Table 4).

ULTRAVIOLET METHOD

The proposed UV method allowed a rapid and economical quantification of PASS in PASS-EXT without the need for time-consuming sample preparation. Moreover, the spectrophotometric methods involve much simpler instrumentation compared with other similar techniques. The absorption spectra of PASS and MAP in aqueous solution is shown in Figure 4. Three maxima peaks were found λmax = 265 nm, 213 nm, and 303 nm, and this last one (303) was selected for all the measurements.

In order to analyze the specificity of the UV method, we studied the possibility to determine the presence of MAP, the degradation product, in the ethanol solution with PASS, and we did not observe peaks at 303 nm corresponding to MAP neither the components of the formulation.

Calibration curves were obtained in the range of expected concentration of PASS (6.37 and 9.88 µg/mL). Beer's law com-

TABLE 1. Results of Regression Analysis of Data for Determination of PASS and MAP in Powder for Reconstitution by HPLC and UV Methods.

STATISTICAL PARAMETERS	HPLC		UV
	PASS	MAP	PASS
Regression equation[a]	$y_1^a = 0.00145x_1^c + 0.0386$	$y_2^b = 1.044x_2^c - 0.0345$	y = 0.040x -0.00759
Correlation coefficient (r)	0.999	0.999	0.999
Standard error of slope	7.00×10^{-6}	0.014	6.063×10^{-4}
Standard error of intercept	5.00×10^{-3}	0.086	4.980×10^{-3}
Concentration range (µg/mL)	483-897	0.700-9.880	5.880-10.920

a) y_1 is the relation peak area IS/PASS; b) y_2 is the relation peak area IS/MAP; c) x_1 and x_2 is the concentration of PASS and MAP in µg/mL.
HPLC = high-performance liquid chromatographic; UV = ultraviolet

TABLE 2. Results of the Determination of PASS and MAP in Powder for Reconstitution by HPLC and UV Methods.

METHOD	SAMPLE	EXPERIMENTAL AMOUNT[c]	PURITY (%)	RSD (%) INTRA-DAY	RSD (%) INTER-DAY[d]
HPLC[a]	MAP 1.2	1.19 (0.41%)	99.12	0.47	0.95
		1.18 (0.35%)	98.75		
		1.20 (0.45%)	100.10		
		1.19 (0.37%)	99.20		
		1.19 (0.32%)	99.18		
		1.19 (0.29%)	99.65		
	PASS 690	683.79 (0.65%)	99.1	0.79	1.23
		692.07 (0.49%)	100.3		
		686.55 (0.39%)	99.5		
		685.17 (0.52%)	99.3		
		686.55 (0.42%)	99.5		
		698.28 (0.66%)	101.2		
UV[b]	PASS 2.9	2.95 (1.72%)	98.8	0.84	2.24
		2.91 (0.34%)	99.1		
		2.88 (0.68%)	98.5		
		2.91 (0.34%)	99.3		
		2.89 (0.34%)	99.5		
		2.94 (1.37%)	99.3		

[a]RP-HPLC: Sample and Experimental amount µg/mL; [b]UV: Sample and Experimental amount µg/mL; [c]Mean of three determinations RSD are listed in brackets; [d]3 days.
HPLC = high-performance liquid chromatographic; RSD = relative standard deviation; UV = ultraviolet

plied over this range, and the representative regression equation was:

$$y = 0.040x - 0.00759$$

with a correlation coefficient of 0.999 (Table 1).

The LOD and LOQ were found to be 0.05 and 0.15 µg/mL, respectively. The validity of the assay was verified by means of the ANOVA. According to this test, there is linear regression ($F_{calculated} > F_{critical}$; P=0.01) and there was no deviation from linearity.

Table 2 shows the experimental values obtained for the PASS determination in different samples, indicating satisfactory intra-day variability (RSD 0.84%) and inter-day variability (RSD 2.24%).

A good accuracy of the method was verified with a recovery of 102.84 ±0.86 (Table 3). The method was found to be robust when the operator was varied.

STABILITY OF SSP

Figure 5 shows the stability profile at 5°C and 25°C. The results were expressed as percentage of MAP produced. The data obtained showed that the degradation product in sample solutions was less that 0.25% when stored at 5°C (0.04%) and 25°C (0.22%) for 24 hours, respectively.

CONCLUSION

A stability-indicating RP-HPLC method was developed and validated for the determination of PASS in the new pharmaceutical formulation developed, a powder for reconstitution. The method was found to be simple, rapid, precise, accurate, and sensitive. Therefore, it is suitable not only for routine quality control, but also for chemical stability studies in the formulation.

The UV method was suitable only for the quantification of PASS in the formulation, being an economic and simple technique with less time-consuming sample preparation for routine assays.

It is still not evident whether the design and development of more advanced anti-TB medicines will benefit a wide sector of the infected population or only the more affluent groups of patients in developed countries. Overall, the challenges ahead demand the design of formulations that address the different limitations of the anti-TB pharmacotherapy and, in addition, make them affordable to all patients, regardless of their socio-economic status. Moreover, it could address the major problem of orphan patients owing to the shortage of the medicine.

TABLE 3. Values Obtained in the Recover Test for PASS and MAP in Powder for Reconstitution by HPLC and UV Methods.

METHOD	ASSAY	SAMPLE CONCENTRATION (g/ML)	% RECOVERY[a] ± (RSD)
HPLC	MAP	1.4	99.25 ± 0.36
		4.24	100.44 ± 0.48
		8.47	100.83 ± 0.38
	PASS	552	100.68 ± 0.66
		690	99.76 ± 0.74
		828	100.19 ± 0.59
UV	PASS	7.2	102.25 ± 1.59
		8.4	102.44 ± 0.80
		9.6	103.83 ± 0.81

[a]Mean of three determinations.
HPLC = high-performance liquid chromatographic; RSD = relative standard deviation; UV = ultraviolet

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FIGURE 4. UV spectrum of a) PASS.2H_2O, b) MAP in ethanol.

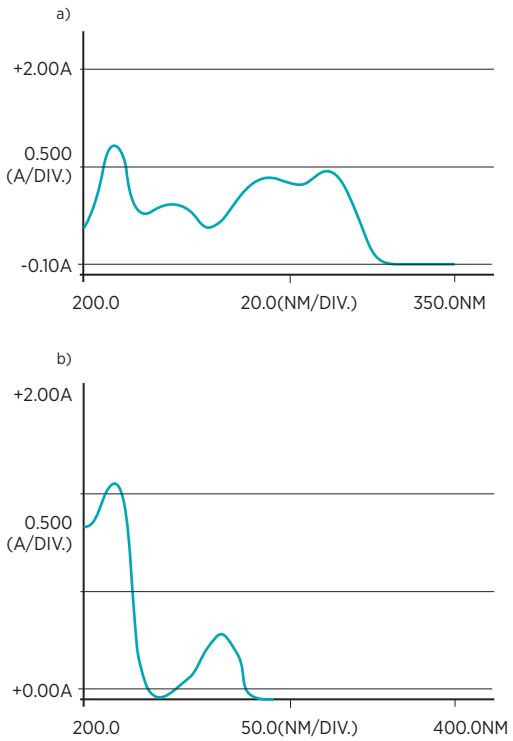

TABLE 4. Values Obtained in the Test of Robustness for MAP and PASS-EXT by the RP-HPLC Method.

ASSAY	SAMPLE (g/ML)	% RECOVERY ± (RSD)	CHANGE OF VARIABLE
MAP	4.24	99.25 ± 0.36	Operator
	4.24	100.44 ± 0.48	Ionic pair
	4.24	100.83 ± 0.38	Instrumental
PASS	690	100.68 ± 0.66	Operator
	690	99.76 ± 0.74	Ionic pair
	690	100.19 ± 0.59	Instrumental

RSD = relative standard deviation; RP-HPLC = reversed phase-high-performance liquid chromatographic

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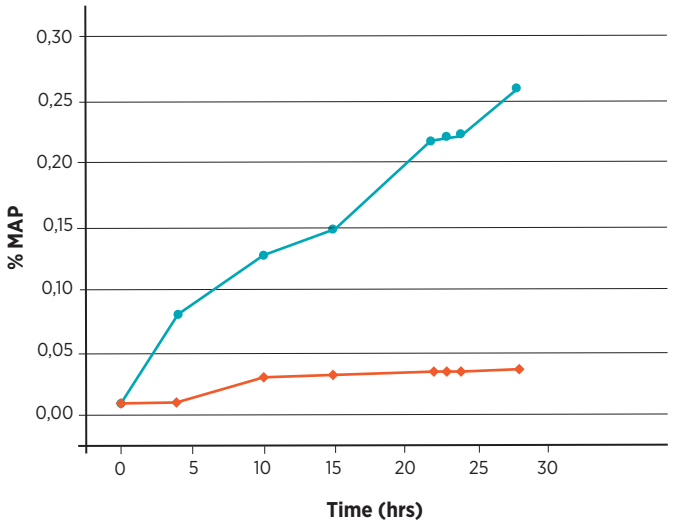
FIGURE 5. Profile of degradation of p-aminosalicylate of sodium in mobile phase stored at 5°C (-♦-) and 25°C (-●-) during 24 hours.



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