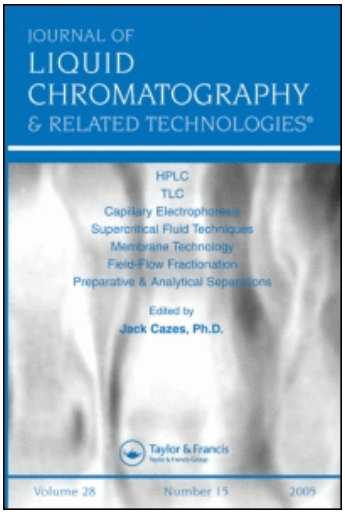


Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

Determination of p-Aminobenzoic and Salicyclic Acid Salts in Pharmaceuticals by High Performance Liquid Chromatography

M. Carlson[a]; R. D. Thompson[a]
[a] U. S. Food and Drug Administration, Pharmaceutical Analysis Section, Minneapolis, Minnesota

To cite this Article Carlson, M. and Thompson, R. D.(1987) 'Determination of p-Aminobenzoic and Salicyclic Acid Salts in Pharmaceuticals by High Performance Liquid Chromatography', Journal of Liquid Chromatography & Related Technologies, 10: 5, 997 — 1009
To link to this Article: DOI: 10.1080/01483918708066749
URL: http://dx.doi.org/10.1080/01483918708066749

JOURNAL OF LIQUID CHROMATOGRAPHY, 10(5), 997-1009 (1987)

DETERMINATION OF p-AMINOBENZOIC AND SALICYLIC ACID SALTS IN PHARMACEUTICALS BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

M. Carlson and R. D. Thompson
U. S. Food and Drug Administration
Pharmaceutical Analysis Section
Minneapolis, Minnesota 55401

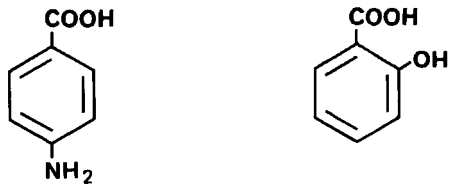
ABSTRACT

A high-performance liquid chromatographic procedure for the analysis of pharmaceutical formulations containing the sodium or potassium salts of p-aminobenzoic acid in combination is described. Replicate analysis of fifteen commercial products gave precision (CV) values having a range of 0.27% to 2.33%. Recovery values obtained from a synthetic mixture formulated at three potency levels for each compound varied from 98.6 to 101.0%, while recoveries from nine fortified products ranged from 97.7% to 101.7%. The detector response at 300 nm for these compounds was observed to be linear over a 100-fold range in concentration. The proposed method is simple, sensitive and specific with respect to spectrophotometric and titrimetric procedures and can be applied to most pharmaceutical products containing these two ingredients.

INTRODUCTION

The alkali salts of p-aminobenzoic acid (PABA) and salicylic acid (SA) shown in Fig. 1 have been used for several decades as analgesic preparations for the treatment of mild to moderate pain associated with rheumatoid arthritis. p-Aminobenzoic acid has also been prescribed for various dermatological ailments including Peyronie's disease, scleroderma, dermatomyositis and as a sunscreen agent. Analgesic products containing the potassium salts of these acids have recently appeared on the market as a result of low-sodium dietary intake requirements. At the present time, there are numerous products available in several dosage forms containing the sodium or potassium salts of these compounds which range in dosage level from 50 mg to 650 mg.

A search of the chemical literature revealed a paucity of methodology for the analysis of the salts of these acids formulated in combination. Current compendial procedures based on titrimetry or UV spectrophotometry apply only to the bulk drug or single component formulations (1). Colorimetric methods have been reported for the assay of PABA using the classical Bratton-Marshall reaction (2-4). Column chromatography on Celite adsorbant has been applied to the mixture but is relatively time-consuming (5). More recently, attention has been focused on



PABA SA

FIGURE 1. Structure of compounds of interest: p-aminobenzoic acid (PABA) and salicylic acid (SA).

the adaptation of high performance liquid chromatography (HPLC) to the separation of related compounds of this class including salicylic acid and benzoic acid (6-8). Isolation was accomplished in these cases with reversed-phase octadecylsilane packings and acidified mobile phases (e.g., ion-suppression). Compendial methodology based on this HPLC technique using a phenylsilane packing has been applied to a single ingredient product in gel form containing PABA with saliylic acid serving as the internal standard (9). An official assay for salicylic acid in a topical foam by HPLC using ion-pairing with tetrabutylammonium hydroxide, a phenylsilane packing and benzoic acid as the internal standard has also been reported (10).

In this communication, we report a simple HPLC procedure for the quantification of the alkali salts (or free acids) of PABA and SA formulated alone or in combination utilizing ion-suppression with an octadecylsilane column and analyte detection at 300 nm. The method has been applied to a large number of products and various dosage forms including plain and enteric-coated tablets, capsules and injectables.

MATERIALS AND METHODS

Reagents and Chemicals

Reference materials were obtained from the following sources: p-aminobenzoic acid (Sigma Chemical Co., St. Louis, MO), potassium and sodium p-aminobenzoate (Ganes Chemical, Inc., Carlstadt, N.J.), salicylic acid (USP Reference Standard), potassium salicylate (Kalama Chemicals, Inc., Seattle, WA), sodium salicylate (Mobay Chemical Corp., New York, NY) and magnesium salicylate (ICN Pharmaceuticals, Inc., Plainview, NY). Methanol (Burdick and Jackson Laboratories, Inc., Muskegon, MI) and monobasic potassium phosphate - KH_2PO_4 (Fisher Scientific Co.,

Fairlawn, NJ) were HPLC grade. All other reagents were analytical reagent grade. Distilled, deionized water passed through a 0.2 micron Versapor membrane filter (Gelman Sciences, Inc., Ann Arbor, MI) was used throughout.

Dilution Solvent

50:47:3 (V/V/V) methanol - water - glacial acetic acid.

Chromatographic System

The HPLC system consisted of an Altex/Beckman Model 100A pump (Beckman Instruments Inc., Berkeley, CA) equipped with a Kratos Model 757 variable wavelength detector (Kratos Analytical Instruments, Ramsey, NJ); a Rheodyne Model 7120 sampling valve having a 20.0 µl fixed loop (Rheodyne Inc., Cotati, CA) and a HP Model 3385A integrator (Hewlett-Packard, Avondale, PA). The column was a 30 cm X 3.9 mm i.d. µBondapak C_{18} - 10 micron (Waters Associates, Milford, MA). The mobile phase was 40:60 methanol/water (V/V) containing 6.8 g/L of monobasic potassium phosphate and adjusted to a pH of 4.0 with 20% (V/V) phosphoric acid. The solution was filtered through a 0.45 micron porosity cellulose acetate membrane (Gelman Sciences, Inc.) prior to use.

Typical operating conditions: mobile phase flow rate 1.0 mL/min, detector at 300 nm, sensitivity 0.01 AUFS, temperature ambient and chart speed 0.5 cm/min. A programmed integrator attenuation change was made at 5.5 min (increase x 4) to allow for differences in the absorptivities of the two compounds.

Standard Solutions

All standard solutions of both the free acids and their respective sodium or potassium salts were prepared at a concentration of 0.005 mg/mL in Dilution Solvent. These solutions were observed to be stable for at least two months when stored

under normal laboratory conditions by comparison to freshly prepared standard solutions.

Procedure

An accurately weighed portion of a finely ground sample composite (20 tablets) equivalent to 100 mg of the active ingredient(s) was transferred to a 500 mL volumetric flask. Fifty milliliters of 3% (V/V) acetic acid in methanol was added and mixed well to wet the sample material followed by 150 mL of Dilution Solvent. The mixture was placed on a mechanical shaker for 30 min and diluted to volume with Dilution Solvent followed by further dilution in a step-wise manner to a final concentration of 0.005 mg/mL for either ingredient with the same solvent. In the case of enteric-coated tablet formulations, the initial extract was sonicated for 1 min prior to mechanical shaking. An additional four-fold dilution for the PABA ingredient and a standard concentration of 0.00125 mg/mL is necessary if an integrator attenuation change is not incorporated into the chromatographic run. Injectable preparations containing sodium salicylate were diluted directly to a concentration of 0.005 mg/mL. An appropriate volume of the diluted sample solution was passed through a 25 mm diameter, 0.45 micron cellulose acetate membrane filter prior to injection of 20.0 µl onto the HPLC column. Quantitation was achieved by comparison of the peak response (area or peak height) obtained to that for the standard solution.

RESULTS AND DISCUSSION

The chromatograms in Fig. 2 show the two compounds to be well resolved with good peak shape and free of interferences (e.g., product excipients) for the sample extracts. This separation is achieved under chromatographic conditions based on ion-suppression

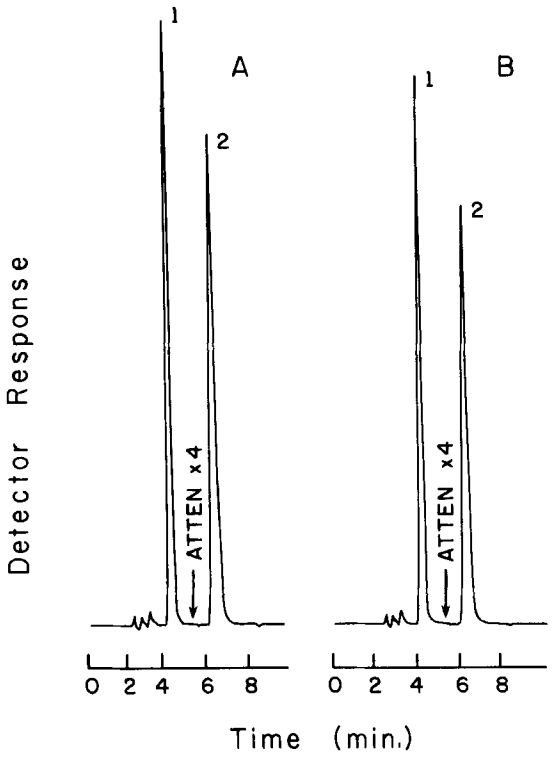


FIGURE 2. A: Chromatograms of a standard solution containing sodium p-aminobenzoate (1) and sodium salicylate (2) and B: an enteric-coated tablet formulation extract. Conditions described under Materials and Methods.

in the reversed-phase mode. The acidic media (pH 4.0) inhibits the dissociation of the carboxyl functions of both compounds in addition to the phenolic moiety of SA. Typical chromatograms shown in Figs. 3 and 4 represent the analysis of single component formulations containing the alkali salts of PABA and SA.

The extracted sample solutions must be passed through membrane material such as cellulose acetate prior to injection. The use of

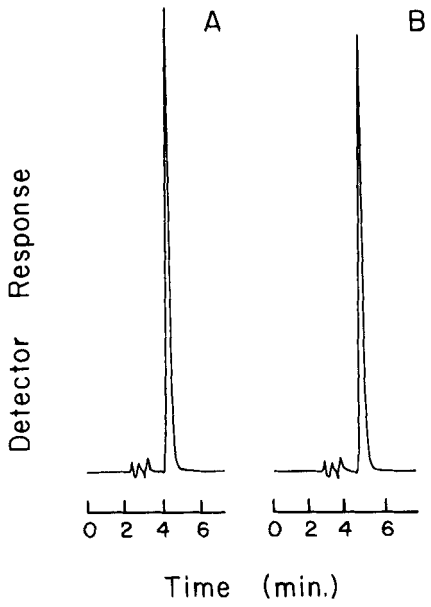


FIGURE 3. Chromatograms of a potassium p-aminobenzoate standard solution (A) and a capsule formulation extract (B). Conditions described under Materials and Methods.

nylon-66 membrane filters resulted in significant adsorption losses of the salicylate component at the working concentration of 0.005 mg/mL employed in the method. This loss was not observed with PABA. Further investigation is being carried out related to this adsorption phenomenon, which we believe may be due to hydrogen-bonding at the nylon membrane surface via the amide functionality. This problem involving SA has not been observed in the presence of polymeric materials such as cellulose acetate and the fluorinated types including polyvinylidene difluoride or PTFE.

The chromatographic response for the two compounds was demonstrated to be linear over at least a 100-fold range in concentration (0.0005 - 0.05 mg/mL) with the minimum detectable

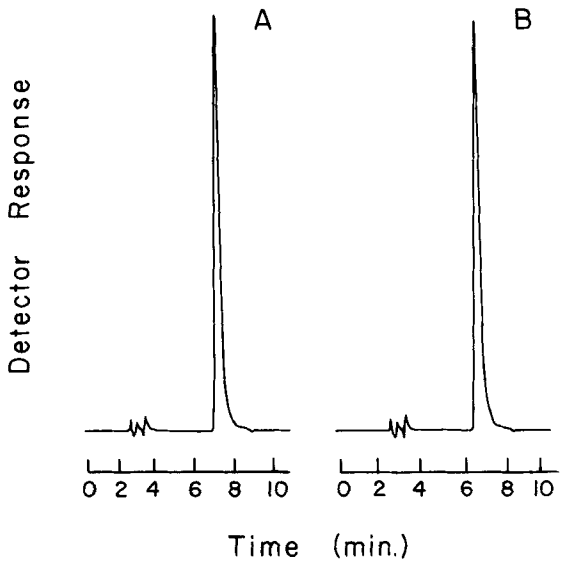


FIGURE 4. Chromatograms of a sodium salicylate standard solution (A) and a plain-coated tablet formulation extract (B). Conditions described under Materials and Methods.

quantity estimated to be Ø.2 ng for potassium p-aminobenzoate and 1 ng for sodium salicylate at a signal-to-noise ratio of 3:1.

A compilation of the assay results and precision values obtained for fifteen commercial formulations representing four different dosage forms is presented in Table 1. Further work involving an inter-laboratory collaborative study of the method is in progress.

Precision

Intra-day precision values for the method outlined in Table 1 expressed as the coefficient of variation (CV) based on five determinations ranged from Ø.38 to 2.33% for the salts (or free

TABLE 1

Multiple Analysis (n=5) of Various Commercial Formulations of p-Aminobenzoic acid (I) and its Salts and the Salts of Salicylic Acid (II)

Product	Compound	Dosage Form	Found wt./dose	% of label claim	CV; %
1	Na-(I)	tablets(E.C.)[A]	0.304 g	101.3	1.06
	Na-(II)		0.300 g	100.0	1.96
2	K-(I)	tablets(P.C.)[B]	0.489 g	97.8	0.44
3	K-(I)	capsules	0.488 g	97.6	0.41
4	(I)	tablets(P.C.)	49.6 mg	99.2	1.60
5	K-(I)	tablets(E.C.)	0.298 g	98.3	0.64
	K-(II)		0.292 g	97.3	0.33
6	(I)	tablets(E.C.)	245.0 mg	98.0	0.53
	K-(II)		277.0 mg	98.9	0.27
7	Na-(II)	tablets(E.C.)	0.326 g	100.6	1.38
8	Na-(II)	tablets(E.C.)	0.659 g	101.7	1.26
9	K-(I)	tablets(P.C.)	0.477 g	95.4	0.47
10	K-(I)	capsules	0.497 g	99.4	0.38
11	K-(I)	tablets(E.C.)	0.324 g	108.0	2.33
	K-(II)		0.328 g	109.3	0.90
12	K-(I)	tablets(E.C.)	0.306 g	102.0	1.86
	K-(II)		0.311 g	103.7	1.50
13	Na-(I)	tablets(E.C.)	0.314 g	104.7	1.53
	Na-(II)		0.316 g	105.3	1.65
14	Na-(I)	tablets(E.C.)	0.308 g	102.3	0.66
	Na-(II)		0.304 g	101.3	1.37
15	Na-(II)	injection	1.032 g	103.2	1.87

[A] Enteric-coated
[B] Plain coated

acid) of PABA and 0.27 to 1.96% for the salts of SA. Inter-day precision was also assessed by analyzing one tablet formulation over a period of five consecutive days. The CV values obtained were 1.57% for sodium p-aminobenzoate and 2.27% for sodium salicylate. In general, higher CV values were observed for SA and its salts based on replicate sample and standard data. This may be due, in part, to the presence of a small amount of peak tailing exhibited for this later eluting species.

Accuracy

Recovery data was obtained by fortifying nine of the fifteen commercial formulations included in this study. These values are presented in Table 2 with recoveries for PABA and its salts ranging from 97.8 to 101.7% and 97.7 to 101.0% for the salts of SA. The accuracy of the procedure was also evaluated by the use of a synthetic preparation based on an enteric-coated tablet formulation. The placebo mixture was formulated at three potency levels with the sodium salts of PABA and SA. The overall results (Table 3) varied from 99.2 to 100.9% for the PABA salt and 98.6 to 101.0% for the SA salt.

Additional Applications

Commercial formulations containing magnesium salicylate are also applicable to this method. Replicate analyses (n=5) of a tablet preparation containing 500 mg of this ingredient gave a CV value of 0.50%. A portion of the product fortified with 100 mg of magnesium salicylate yielded a recovery of 102.6%. A dietary supplement containing 50 mg of PABA was also assayed by the method (Table 1, Product 4). An antibacterial-analgesic tablet formulation containing sodium salicylate, salicylamide, methenamine and benzoic acid was analyzed which resulted in a single chromatographic peak at 7.1 min due to the salicylate and salicylamide. The separation of these two species was readily

TABLE 2

Recovery Data from Fortified Commercial Formulations for p-Aminobenzoic Acid (I) and its Salts and the Salts of Salicylic Acid (II), (n=1)

Product	Compound	Dosage Form	Added	Recovered	% Recovery
1	Na-(I)	tablets(E.C.)[A]	174.0 mg	170.2 mg	97.8
	Na-(II)		174.0 mg	171.1 mg	98.3
2	K-(I)	tablets(P.C.)[B]	199.5 mg	199.1 mg	99.8
3	K-(I)	capsules	151.7 mg	151.3 mg	99.7
4	(I)	tablets(P.C.)	50.3 mg	49.9 mg	99.2
5	K-(I)	tablets(E.C.)	151.7 mg	152.5 mg	100.5
	K-(II)		150.4 mg	151.9 mg	101.0
6	(I)	tablets(E.C.)	101.6 mg	103.3 mg	101.7
	K-(II)		119.4 mg	119.9 mg	100.4
7	Na-(II)	tablets(E.C.)	120.2 mg	119.9 mg	99.8
8	Na-(II)	tablets(E.C.)	119.9 mg	117.7 mg	97.7
9	Na-(II)	injection	100.1 mg	99.2 mg	99.1

[A]Enteric-coated
[B]Plain coated

achieved by adjusting the pH of the mobile phase to 4.5. The retention times for the salicylate and the salicylamide were 5.5 min and 6.8 min under these conditions. Neither methenamine nor benzoic acid were detectable at the monitoring wavelength of 300 nm. However, benzoic acid can also be assayed in the formulation by employing a detection wavelength of 230 nm. The utility of the method might also be extended to sunscreen preparations containing PABA or its esters.

TABLE 3

Recovery of Sodium p-Aminobenzoate (I) and Sodium Salicylate (II) from Synthetic Formulations.[A]

Compound	Recovery, % Formulated at 80% level	100% level	120% level
I	99.2	100.9	99.2
II	98.6	101.0	99.8

[A]Formulated with: Calcium Carbonate, Calcium Sulfate, D&C yellow No. 10, FD&C yellow No. 6, Magnesium Stearate, Stearic Acid and Sucrose.

ACKNOWLEDGEMENT

The authors wish to acknowledge Dr. Charles Carlin for critical review of the manuscript, and Curtis Leach and Krista Baas for preparation of the manuscript.

REFERENCES

1. U.S. Pharmacopoeia, 21st rev., U.S. Pharmacopoeial Convention, Inc. Rockville, MD, (1985), pp 37-39, 951, 958.

2. Bratton, A.C. and Marshall, E.K., A New Coupling Component for Sulfanilamide Determination, J. Biol. Chem., 128, 537 (1939).

3. Nakamura, T., Ueda, T. and Tanaka, K., Mechanisms of Monofluoro acetanilide Poisoning in Mammals IV Micro-determination of Substituted Anilines in Biological Material, J. Agric. Chem. Soc. Japan, 40 13 (1966).

4. Balica, G. and Parvanescu, H., Spectrophotometric Determination of Aminobenzoic Acid (Vitamin H), Rev. Roum. Chim., 25 1013 (1974).

5. Thompson, R.D., Analysis of Formulations Containing p-Aminobenzoic Acid (PABA) and Salicylic Acid Salts, Laboratory Information Bulletin No. 1514, U.S. Food and Drug Admin. (1972).

6. Das Gupta V., Quantitative Determination of Benzoic Acid and Salicylic Acid in Ointments by High-Pressure Liquid Chromatography, J. Pharm. Sci., 66, 110 (1977).

7. Narang, P.K., Bird, G. and Crouthamel, W.G., High-Performance Liquid Chromatographic Assay for Benzocaine and Aminobenzoic Acid Including Preliminary Stability Data, J. Pharm. Sci., 69, 1384 (1980).

8. Collinge, A. and Noirfalise, A., Determination of Seven Food Preservatives by High-Performance Liquid Chromatography, Anal. Chim. Acta, 132, 201 (1981).

9. U.S. Pharmacopoeia, 21st rev., U.S. Pharmacopoeial Convention, Inc., Rockville, MD, (1985), p. 39.

10. Idem., p.952.