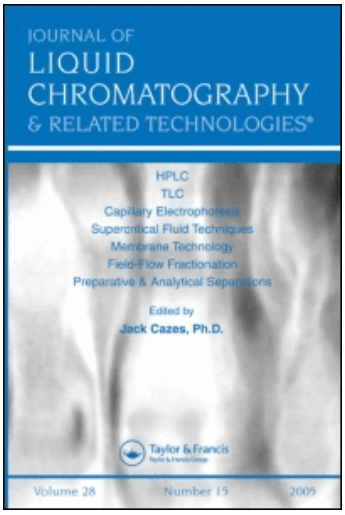

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

An HPLC Method for the Determination of Vitamin B1, Caffeine, Acetylsalicylic Acid, and The Impurities of Salicylic Acid in a Pharmaceutical Preparation

L. Gámiz-Gracia[a]; M. D. Luque de Castro[a]
[a] Department of Analytical Chemistry Faculty of Sciences, University of Córdoba, Córdoba, Spain

To cite this Article Gámiz-Gracia, L. and de Castro, M. D. Luque(1997) 'An HPLC Method for the Determination of Vitamin B1, Caffeine, Acetylsalicylic Acid, and The Impurities of Salicylic Acid in a Pharmaceutical Preparation', Journal of Liquid Chromatography & Related Technologies, 20: 13, 2123 — 2133
To link to this Article: DOI: 10.1080/10826079708005570
URL: http://dx.doi.org/10.1080/10826079708005570

J. LIQ. CHROM. & REL. TECHNOL., 20(13), 2123–2133 (1997)

AN HPLC METHOD FOR THE DETERMINATION OF VITAMIN B1, CAFFEINE, ACETYLSALICYLIC ACID, AND THE IMPURITIES OF SALICYLIC ACID IN A PHARMACEUTICAL PREPARATION

L. Gámiz-Gracia, M. D. Luque de Castro*

Department of Analytical Chemistry
Faculty of Sciences
University of Córdoba
E-14004 Córdoba, Spain

ABSTRACT

An HPLC method for the determination of Vitamin B1, caffeine, acetylsalicylic acid and salicylic acid in tablets containing all four drugs is reported. After optimization of the variables involved, the method has been characterized and validated in terms of calibration (Standard Addition Methodology), repeatability and selectivity, and finally, applied to the quality control of the final product.

INTRODUCTION

Calmante Vitaminado is a Spanish pharmaceutical with analgesic effects for mild and moderate pain, which contains acetylsalicylic acid (ASA), caffeine and vitamin B1 as active compounds. In addition, salicylic acid (SA) is present as an impurity, while wheat and magnesium stearate are found as excipients in the tablets. Despite the fact that methods for the individual determination of

these compounds in mixtures have been reported so far, there are no methods for the joint determination of these three active drugs. Various HPLC methods for the determination of thiamine, together with other water-soluble and lipo-soluble vitamins in multivitamin preparations, can be found in the analytical literature.[1-4] Pharmaceuticals containing ASA and caffeine in binary or higher multianalgesic mixtures are very common, so a pleiade of methods, mainly based on HPLC,[5-9] have been reported so far. Nevertheless, there are no methods in the pharmaceutical literature for the determination of ASA, caffeine, and vitamin B1.

We have developed and validated an HPLC method for the joint determination of the active compounds of Calmante Vitaminado, in order to use it for the quality control of the final product (tablets). As, salicylic acid is common in formulations containing ASA as a result of both its presence as an impurity of SAS and its formation during the preparation of aspirin products,[10] the method also involves the determination of SA.

MATERIALS

Reagents

HPLC-grade methanol (Scharlau Chemicals), orthophosphoric acid (85% w/w, Merck), potassium dihydrogen phosphate (Merck), and ultrapure water were used to prepare the HPLC mobile phase. Standard solutions were prepared from Vitamin B1, caffeine, acetylsalicylic acid, magnesium stearate, wheat starch (kindly provided by Calmante Vitaminado, S.A., Spain), and salicylic acid (Aldrich). Albet filter-paper (No 1305) was used for sample preparation.

Apparatus and Instruments

The HPLC system consisted of a Knauer HPLC pump-64, a Rheodyne 7125 high-pressure injection valve furnished with a 135 μL loop, a Waters 490 programmable multiwavelength uv/vis detector, and a Knauer recorder. The 250×4.6 mm i.d. column was packed with 5 μm Ultrabase C_{18} (Scharlau Science, Spain). A Bandelin Sonorex K 52 ultrasonic bath was used to dissolve the samples.

METHODS

Chromatographic Conditions

The mobile phase was methanol-0.02 M potassium dihydrogen phosphate (30:70, v/v) (pH 4.0) at a flow-rate of 1.5 mL min^{-1}. The injection volume was 135 μL and the UV detector was operated at 246 nm (vitamin B1), 274 nm (caffeine), and 224 nm (acetylsalicylic acid and salicylic acid). These wavelengths provided the maximum absorbance for each compound. The retention times of vitamin B1, caffeine, SA and SAS acid were 2.3, 6.6, 9.7, and 12.8 min, respectively. Figure 1 shows a typical HPLC chromatogram.

Preparation of the Mobile Phase

A 0.02 M buffer solution was prepared from potassium dihydrogen phosphate and ultrapure water. HPLC grade methanol and the buffer solution, filtered through a 0.45 μm Nylon filter, were mixed in a 30:70 (v/v) ratio. The solution was degassed in an ultrasonic bath for 15 min and the pH adjusted to 4.0 by adding orthophosphoric acid (1% in ultrapure water).

Preparation of the Standard Solutions

Calibration solutions were prepared by diluting the standard stock solution (100 mg l^{-1} of each compound prepared in the mobile phase) with the mobile phase. The solutions were sonicated for 5 min.

Preparation of the Tablet Solutions and Procedure

10 tablets accurately weighed were ground and mixed in a mortar. 75 mg of the powder was transferred to a 25 mL flask and made to volume with mobile phase, sonicated for 15 min, and filtered through filter-paper.

For vitamin B1 determination, the solution was injected directly, whereas for the determination of the other compounds, a dilution (200 μL in 10 mL) with the mobile phase was necessary. Duplicate injections were performed in all instances. Measurements were based on peak height.

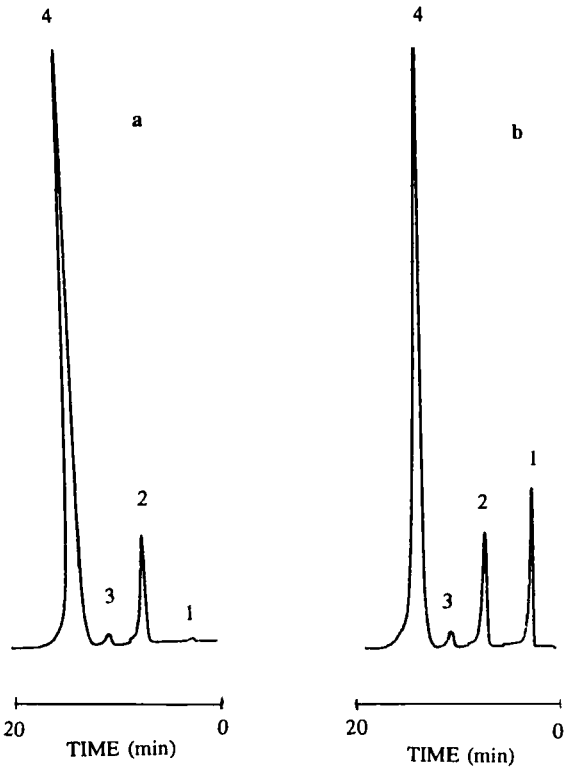

Figure 1. HPLC chromatogram **(a):** Tablet solution (60 μg mL^{-1}). **(b):** Tablet solution (60 μg mL^{-1}) "spiked" with Vitamin B1 (10 μg mL^{-1}). Peak identification: (1): Vitamin B1; (2): caffeine; (3): salicylic acid; (4): acetylsalicylic acid. See text for chromatographic conditions.

RESULTS AND DISCUSSION

Optimization of the Chromatographic Conditions

As the vitamin B1-caffeine-ASA ratio in the tables is 1:25:250 (by weight), the optimization of the chromatographic conditions was carried out using similar amounts of the three active compounds, as well as of SA in order to clearly distinguish the behaviour of each of the compounds whilst changing the variables.

The experimental variables, optimized to obtain adequate separation of the eluted analytes, were the composition of the mobile phase, the flow-rate, and the injection volume.

The influence of the percentage of methanol in the binary methanol-phosphate buffer mixtures used as mobile phase to separate the analytes in a Ultrabase C_{18} column, was studied in the range 0-100% v/v methanol. A 30:70 methanol-buffer mixture at pH 4.0 was selected as optimal.

The influence of the flow-rate of the mobile phase was studied in the range 0.5-2.0 mL min^{-1}, providing an optimum value at 1.5 mL min^{-1}.

An injection volume of 135 μL was selected as optimum. Above this value, the increase in sensitivity did not compensate for the increase in pressure.

A mixture of the analytes at the concentration ratio in the tablets was injected after optimization. Figure 1 shows the cromatogram obtained, which justified the use of two injections of the sample at different dilutions.

Validation of the Method

Calibration

The standard addition methodology[11] was used for calibration. The sets of data obtained in 3 calibration experiments with standard solutions (namely: Standard Calibration, SC), standard additions (Standard-addition calibration, AC), and portions of sample (Youden Calibration, YC) were used for each compound. The accuracy of the analytical results was checked by comparing both the analytes content in the different calibrations and the recoveries, calculated by dividing the net content found by that added for each addition. The Alamin program was used for calculation.[12]

The SC were run with triplicate injections of the standard solutions and the responses versus concentrations were linear in the following ranges:

Vitamin B1	1.9-25 mg L^{-1}
Caffeine	2.2-25 mg L^{-1}
Salicylic acid	1.5-25 mg L^{-1}
Acetylsalicylic acid	3.6-50 mg L^{-1}

Table 1

Features of the Proposed HPLC Method for the Determination of Vitamin B1, Caffeine, Salicylic Acid, and Acetylsalicylic Acid

	r^2(%)	s_{xy}	s_a	s_b	Calibration Curve
Vitamin B1					
SC	99.91	$2.16 \cdot 10^{3}$	$9.02 \cdot 10^{-4}$	$5.96 \cdot 10^{-5}$	$Y=8.57 \cdot 10^{-4}+8.08 \cdot 10^{-3} \cdot X$
AC	99.28	$5.91 \cdot 10^{-3}$	$4.94 \cdot 10^{-3}$	$5.28 \cdot 10^{-4}$	$Y=55.30 \cdot 10^{-3}+8.76 \cdot 10^{-3} \cdot X$
YC	99.86	$1.73 \cdot 10^{-3}$	$2.12 \cdot 10^{-3}$	$8.00 \cdot 10^{-7}$	$Y=1.00 \cdot 10^{-3}+2.90 \cdot 10^{-5} \cdot X$
Caffeine					
SC	99.89	$2.24 \cdot 10^{-3}$	$9.35 \cdot 10^{-4}$	$6.18 \cdot 10^{-5}$	$Y=-1.43 \cdot 10^{-4}+7.30 \cdot 10^{-3} \cdot X$
AC	99.86	$2.31 \cdot 10^{-3}$	$1.94 \cdot 10^{-3}$	$2.07 \cdot 10^{-4}$	$Y=60.60 \cdot 10^{-3}+7.82 \cdot 10^{-3} \cdot X$
YC	99.99	$6.32 \cdot 10^{-4}$	$7.75 \cdot 10^{-4}$	$5.70 \cdot 10^{-6}$	$Y=-3.00 \cdot 10^{-3}+7.16 \cdot 10^{-4} \cdot X$
Salicylic Acid					
SC	99.95	$1.50 \cdot 10^{-3}$	$6.26 \cdot 10^{-4}$	$4.13 \cdot 10^{-5}$	$Y=-1.17 \cdot 10^{-3}+7.10 \cdot 10^{-3} \cdot X$
AC	99.96	$1.16 \cdot 10^{-3}$	$9.72 \cdot 10^{-4}$	$1.04 \cdot 10^{-4}$	$Y=29.40 \cdot 10^{-3}+6.98 \cdot 10^{-3} \cdot X$
YC	1.00	0	0	0	$Y=-1.0 \cdot 10^{-3}+8.00 \cdot 10^{-5} \cdot X$
Acetylsalicylic Acid					
SC	99.92	$3.16 \cdot 10^{-3}$	$1.32 \cdot 10^{-3}$	$4.37 \cdot 10^{-5}$	$Y=-3.03 \cdot 10^{-3}+6.10 \cdot 10^{-3} \cdot X$
AC	99.95	$2.14 \cdot 10^{-3}$	$1.79 \cdot 10^{-3}$	$9.59 \cdot 10^{-5}$	$Y=68.40 \cdot 10^{-3}+6.04 \cdot 10^{-3} \cdot X$
YC	99.99	$3.87 \cdot 10^{-4}$	$4.74 \cdot 10^{-4}$	$1.73 \cdot 10^{-5}$	$Y=-5.00 \cdot 10^{-4}+3.43 \cdot 10^{-3} \cdot X$

SC: Standard calibration
AC: Standard addition calibration
YC: Youden calibration
Y = Absorbance (peak height)
X = Concentration in $\mu g\ mL^{-1}$

Only one injection of each solution was made in the two other calibration procedures. The numerical values of the parameters of these calibrations are shown in Table 1. The values of the slopes (SC and AC) are similar in all instances.

Table 2

Results of Recovery Assays (from the Standard-Addition Calibration) to Check Accuracy

Vitamin B1[a]	Sample [b]	R[c] (%)	R[c] (Average)
5	2	98.40	
10	2	93.43	99.73
15	2	107.35	
Caffeine[b]	**Sample[a]**		
5	100	89.45	
10	100	103.23	98.66
15	100	103.29	
Salicylic Acid	**Sample[a]**		
5	400	97.12	
10	400	100.73	98.99
15	400	99.11	
Acetylsalicylic Acid[a]	**Sample[a]**		
10	20	98.11	
20	20	98.51	98.78
30	20	99.73	

[a] In $\mu g\ mL^{-1}$
[b] In $mg\ mL^{-1}$
[c] Recovery

The results from SC and AC are not significantly different, so the method is accurate. The average recoveries from the AC are shown in Table 2. This supports the accuracy of the method. The conclusion obtained is that the determination of the four compounds in tablets can be carried out directly by the SC methods. The figures of merit of the method, calculated from SC data sets,[13] are shown in Table 3.

Table 3

Features of the Analytical Method from the Standard Calibration Data Set

	Vitamin B1	Caffeine	Salic. Acid	Acetylsalic. Acid
Sensitivity[a]	0.267	0.306	0.211	0.512
Precision (RSD, %)	1.13[b]	1.25[b]	0.88[b]	1.08[c]
Detect. limit[a]	0.572	0.655	0.449	1.090
Determ. limit[a]	1.908	2.182	1.498	3.632
Linearity (%)	99.26	99.16	99.42	99.29

[a] In $\mu g\ mL^{-1}$
[b] At $15 \mu g\ mL^{-1}$
[c] At $30\ \mu g\ mL^{-1}$

Selectivity

The excipients of the commercial tablets (magnesium stearate and wheat starch) caused no effect in the determination of the compounds, as they did not absorb in the uv region where measurements were performed, and they were not retained by the column, so the determination of the active compounds of this pharmaceuticals is free from interferences.

Reproducibility

The method was applied, under the optimal working conditions, to 10 samples from powder obtained from 10 commercial tablets, and injected in duplicate. The samples were prepared as described previously. The results are shown in Table 4. The highest RSD value corresponds to the analyte at lower concentration (SA: RSD 12%); while more acceptable RSDs are obtained for ASA and vitamin B1 (note that a more concentrated sample is used for the latter).

Application of the Method

The performance of the method was tested by applying it to the determination of the target compounds in 12 samples each obtained from 10 tablets of 12 different batches of commercial tablets.

Table 4

Reproducibility of the Method: Results from 10 Different Samples, Injected in Duplicate

	Retention Time (Min)	Mg/Tablet
Vitamin B1	2.29±0.06 (RSD=2.52%)	2.17±0.09 (RSD=3.96%)
Caffeine	6.63±0.13 (RSD=1.94%)	59±4 (RSD=6.63%)
Salicylic Acid	9.39±0.17 (RSD=1.76%)	13.9±1.7 (RSD=12.11%)
Acetylsalicylic Acid	12.58±0.16 (RSD = 1.27%)	446±11 (RSD = 2.47%)

Table 5

Application of the Proposed Method to the Analysis of Tablets from Different Batches, Injected in Duplicate

Batch Number	Vitamin B1		Caffeine		Salicyclic Acid		Acetylsalicylic Acid	
	mg/Tablet	t_r	mg/Tablet	t_r	mg/Tablet	t_r	mg/Tablet	t_r
1	2.17	2.29	59.09	6.63	13.95	9.39	445.93	12.58
2	2.23	2.22	63.09	6.60	12.02	9.85	517.07	12.58
3	2.24	2.31	46.71	6.70	9.23	10.08	316.16	13.05
4	2.35	2.36	53.82	6.57	7.51	10.08	392.05	12.72
5	2.53	2.34	57.70	6.63	8.70	10.13	466.22	13.15
6	3.23	2.26	64.39	6.53	9.06	10.13	495.83	13.07
7	2.66	2.25	55.18	6.62	7.50	10.30	414.99	13.28
8	2.48	2.37	51.92	6.47	8.88	10.19	386.71	12.94
9	3.13	2.34	49.84	6.58	10.47	9.91	409.16	12.97
10	3.22	2.32	47.07	6.72	7.56	10.20	376.17	13.21
11	3.00	2.34	48.92	6.65	9.10	10.06	387.97	13.14
12	3.23	2.32	49.18	6.71	6.18	10.15	337.23	13.21
Average	2.7	2.31	53.9	6.62	9.2	10.04	412.1	12.99
s	0.4	0.05	6.1	0.06	2.1	0.24	60.2	0.24
RSD (%)	15.8	2.0	11.2	1.1	23.2	2.4	14.6	1.9

Samples were prepared as described previously and injected in duplicate. The results are shown in Table 5. The analysis revealed some shortcomings in the manufacturing process, both because the concentrations found did not always agree with the nominal content of the pharmaceutical preparation, and because of the differences between batches.

CONCLUSIONS

The method proposed here does not enable the determination of the four analytes in a single injection owing to the large differences in their concentrations in the target pharmaceuticals. The two-injection determination provides excellent precision for the most dilute active compound (vitamin B1).

The content of the decomposition product of ASA is below the limit permitted by the European Pharmacopeia in ASA-containing tablets (less than 3.2%). The validation study demonstrated the accuracy of the method, which can be safely applied for quality control of the final product.

AKNOWLEDGMENT

The Comisión Interministerial de Ciencia y Tecnología (CICyT) is thanked for financial support (Project No. ABM96-1086).

REFERENCES

1. N. R. Raju, P. Srilakshmi, G. A. Vinolia, G. J. Bhounsule, V. D. Gaitonde, Indian Drugs, **26**, 697-701 (1989).

2. A. Kozik, M. Rapala-Kozik, J. Chomatogr., **648(2)**, 349-356 (1993).

3. M. C. Genaro. J. Chromatogr. Sci., **29(9)**, 410-415 (1991).

4. M. Amin, J. Reusch, J. Chromatogr., **390(2)**, 448-453 (1987).

5. M. A. S. Salem, H. N. Alkaysi, Drug. Dev. Ind. Pharm., **16(7)**, 1185-1201 (1990).

6. M. A. Abuirjeie, M. E. Abdel-Hamid, E. A. Ibrahim, Anal. Lett., **22(2)**, 365-375 (1989).

7. K. K. Verma, S. K. Sanghi, A. Jain, D. Gupta, J. Pharm. Sci., **76(7)**, 551-553 (1987).

8. I. M. Jalal, S. I. Sa'-sa', Talanta, **31(11)**, 1015-1017 (1984).

9. V. Das-Gupta, J. Pharm. Sci., **69(1)**, 110-113, (1980).

10. R. N. Bevitt, J. R. Mather, D. C. Sharman, Analyst, **109(10)**, 1327-1329, (1984).

11. L. Cuadros Rodríguez, A. M. García Campaña, F. Alés Barrero, C. Jiménez Linares, M. Román Ceba, J. A. O. A. C., Int'l., **78(2)**, 471-476 (1995).

12. A. M. García Campaña, J. L. Sierra Fernández, L. Cuadros Rodríguez, F. Alés Barrero, M. Román Ceba, Trends Anal. Chem., submitted for publication.

13. L. Cuadros Rodríguez, A. M. García Campaña, C. Jiménez Linares, M. Román Ceba, Anal. Lett., **26(6)**, 1243-1258 (1993).

Received October 20, 1996
Accepted December 16, 1996
Manuscript 4314