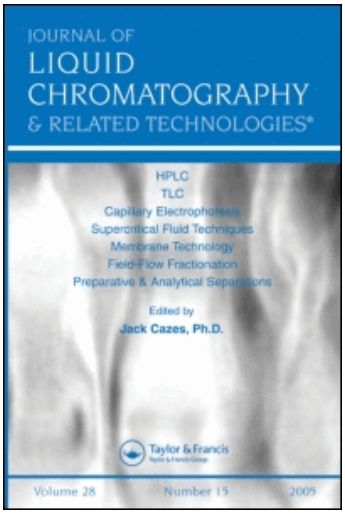

Journal of Liquid Chromatography & Related Technologies

Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

High Performance Liquid Chromatography Simultaneous Quantitation of Ketoprofen and Parabens in a Commercial GEL Formulation

C. Mannucci[a]; J. Bertini[a]; A. Cocchini[a]; A. Perico[a]; F. Salvagnini[a]; A. Triolo[a]
[a] Analytical Research Department, A. Menarini Industrie Farmaceutiche Riunite S.R.L V., Firenze, Italy

To cite this Article Mannucci, C. , Bertini, J. , Cocchini, A. , Perico, A. , Salvagnini, F. and Triolo, A.(1992) 'High Performance Liquid Chromatography Simultaneous Quantitation of Ketoprofen and Parabens in a Commercial GEL Formulation', Journal of Liquid Chromatography & Related Technologies, 15: 2, 327 — 335
To link to this Article: DOI: 10.1080/10826079208017173
URL: http://dx.doi.org/10.1080/10826079208017173

JOURNAL OF LIQUID CHROMATOGRAPHY, 15(2), 327-335 (1992)

HIGH PERFORMANCE LIQUID CHROMATOGRAPHY SIMULTANEOUS QUANTITATION OF KETOPROFEN AND PARABENS IN A COMMERCIAL GEL FORMULATION

C. MANNUCCI, J. BERTINI, A. COCCHINI, A. PERICO, F. SALVAGNINI, AND A. TRIOLO

Analytical Research Department
A. Menarini Industrie Farmaceutiche Riunite s.r.l.
V. Sette Santi 3, Firenze, Italy

ABSTRACT

A simple and rapid procedure for quantitation of Ketoprofen and parabens simultaneously in a commercial gel formulation by reversed phase high performance liquid chromatography was developed. The chromatographic analysis has been undertaken on a Hypersil ODS column by using a solvent mixture composed of acetonitrile-potassium dihydrogenphosphate buffer (pH = 3.0) (40:60, v/v) isocratically with a U.V. detection at 254 nm. Ketoprofen related impurities and hydrolysis product of parabens are well resolved in these conditions and the method can be applied to quality control analysis of Ketoprofen and parabens in gel formulation.

INTRODUCTION

Ketoprofen (2-(3-benzoyl-phenyl)propionic acid) is a non steroidal anti-inflammatory agent[1] of common clinical use through different routes of administration[2-4]. In Menarini laboratories, the pharmaceutical preparation of a gel (Fastum® gel) containing Ketoprofen was developed to obtain anti-inflammatory and analgesic activity in those diseases of the muscolo-skeletal apparatus in which a local action is preferred.[5-7] In gel formulations there are also included substances having the role of preservatives, in order to prevent bacterial growth. In Fastum® gel, a mixture of the esters of p-hydroxy benzoic acid (parabens) has been utilized. For the analysis of such a formulation, a method capable of dosing simultaneously the active drug and the parabens was developed. For quality control purpose, the whole procedure had to be as simple and rapid as possible, and the final determination specific and affidable. High performance liquid chromatography was considered the analytical method most capable of meeting these requirements; in fact it had already been used for the determination of impurities and degradation products [8-9] of Ketoprofen as raw material, and so its use for quality control analyses of a finished product appeared possible.

EXPERIMENTAL

Apparatus:

A high performance liquid chromatograph (Hewlett-Packard model 1090M) equipped with a variable wavelenght diode array detector, a variable volume autoinjector, an autosampler and a thermostatic column oven, interfaced with a work station was used for the analysis.
A stainless steel column packed with Hypersil ODS 5 μm, 150 x 4.6 mm (Alltech Associates,Deerfield,IL) coupled with a guard-column RP-18 7μm, 15 x 3.2 mm (Brownlee Labs,Santa Clara,CA) was used.

The mobile phase consisted of acetonitrile and a solution of potassium dihydrogenphosphate 0.01 M at pH = 3.0 adjusted with phosphoric acid, mixed in ratio of 40:60 v/v. Flow rate was set at 1.2 ml/min, oven temperature at 50°C and injection volume at 5µl. Detector wavelenght was set at 254 nm with a bandwith of 4 nm and a reference wavelenght of 550 nm with a bandwith of 100 nm. In these operative conditions the following capacity factors were obtained:

Methyl-p-hydroxy benzoate	0.69
Ethyl-p-hydroxy benzoate	1.27
Propyl-p-hydroxy benzoate	2.34
Ketoprofen	3.01
Butyl-p-hydroxy benzoate	4.32

A typical chromatogram of standard working solution is shown in fig. 1

System Suitability:

In the described operative conditions the chromatographic system must be in agreement with the following parameters by injecting a mixture of parabens at a concentration of 0.5 mg/ml in methanol.
The minimum number of theoretical plates in chromatographic column, calculated on butyl paraben peak, is ≥ 7000. Resolution between methyl and ethyl paraben peaks is ≥ 7 and between propyl and butyl paraben peaks is ≥ 5. Tailing factor calculated using the butyl paraben peak is ≤ 1.2.

Reference Materials and Chemicals:

Acetonitrile HPLC grade, potassium dihydrogenphosphate, methanol and phosphoric acid analytical grade were obtained from E.Merck (Darmstadt, Germany).
As reference substances were used for Ketoprofen, a working standard prepared by analysing a batch of raw material with respect to an USP standard and for parabens a

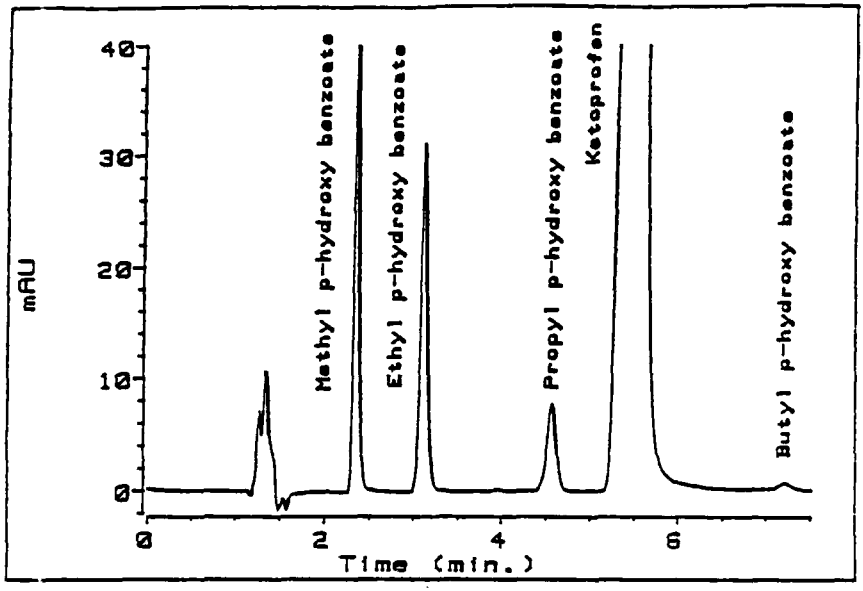

FIGURE 1: Chromatogram of a sample of gel.

commercial mixture named Septocombin® having the following composition were utilized:

Methyl-p-hydroxy benzoate	39.3 %
Ethyl-p-hydroxy benzoate	41.8 %
Propyl-p-hydroxy benzoate	16.4 %
Butyl-p-hydroxy benzoate	2.5 %

Pharmaceutical preparation used for analysis was Fastum® gel, from Menarini Industrie Farmaceutiche Riunite s.r.l., claiming to contain g 2.5 of Ketoprofen and g 0.1 of Septocombin for g 100 of gel.

Standard Solutions:

A stock solution of Ketoprofen was prepared at a concentration of 1000 μg/ml in methanol and another one of

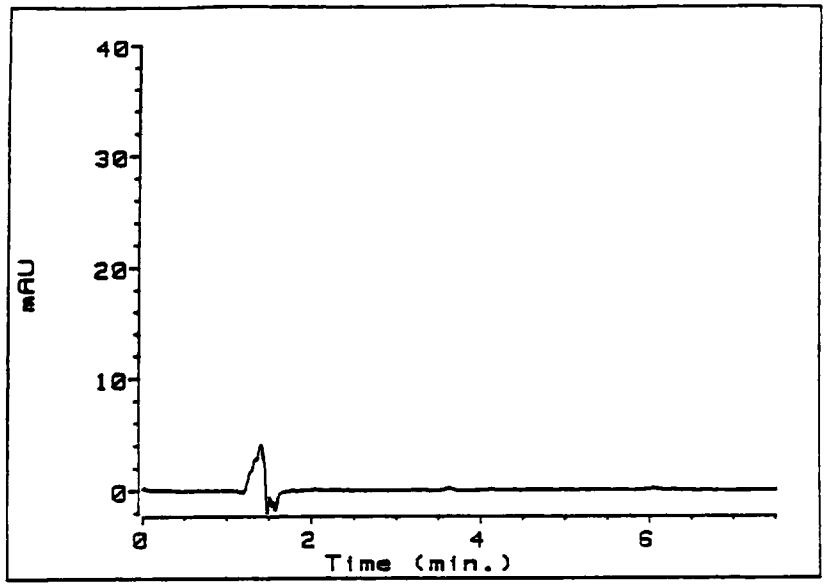

FIGURE 2: Chromatogram of a sample of blank gel.

Septocombin at a concentration of 500 µg/ml in methanol. These stock solutions were stable at room temperature for at least a week.

The working solution of standards was freshly prepared by diluting to 50 ml with methanol 2.0 ml of Septocombin stock solution and 25.0 ml of Ketoprofen stock solution. At least 6 injections of working solution must be made on LC-column to calculate the mean area values of each peak.

Analysis of Gel:

About 500 mg of gel was accurately weighed into a 25 ml calibrated flask and diluted to final volume with methanol. The mixture was heated at 50°C for 10 minutes in a thermostatic bath and then put into a ultrasonic bath for 2 minutes. The suspension was shaken untill room temperature

TABLE 1
Capacity Factors of Bulk Impurities and Break-Down Products Tested.

p-hydroxy benzoic acid	0.16
2-(3-benzoylphenyl)propionamide	1.57
3-benzoylphenyl acetic acid	1.93
2-[3-(4-methylbenzoyl)phenyl]propionic acid	4.90
2-(3-benzoylphenyl)propionitrile	6.42
2-(3-dimethylbenzoyl)phenyl propionic acid	7.66

was reached, centrifuged for 10 minutes at 4000 rpm and then injected (at least 3 runs) onto the LC-column.
To determine the quantities of Ketoprofen and Septocombin in gel formulation, the external standard method was used.

RESULTS AND DISCUSSION

The specificity of method was tested by following the procedure of sample preparation on a blank gel and no interfering peaks were detected in the chromatographic profile (fig. 2).
The known bulk impurities of Ketoprofen and hydrolitic degradation product of parabens under these chromatographic conditions were well separated from the Ketoprofen and Septocombin peaks as shown in table 1.
Response linearity was tested on two different days by injecting different volumes (1-2.5-5-7.5-10 µl) of the working solution of standard and replicating each volume three times. In table 2 are shown the determination coefficients for each component obtained in the study by reporting the mean area values versus the injected volumes.

TABLE 2
Determination Coefficients of Ketoprofen and Parabens

	R^2 (day 1)	R^2 (day 2)
Ketoprofen	1.000	1.000
Methyl-p-hydroxy benzoate	0.984	1.000
Ethyl-p-hydroxy benzoate	0.999	1.000
Propyl-p-hydroxy benzoate	0.998	0.998
Butyl-p-hydroxy benzoate	0.997	0.998

TABLE 3
C.V. of Ketoprofen and Parabens calculated injecting 6 replicates of working solution of standards and a sample solution.

	C.V. (day 1)	C.V. (day 2)
Ketoprofen	0.10% (a) 0.86% (b)	0.38% (a) 1.47% (b)
Methyl-p-hydroxy benzoate	0.41% (a) 2.58% (b)	1.07% (a) 1.41% (b)
Ethyl-p-hydroxy benzoate	0.79% (a) 0.48% (b)	0.28% (a) 0.88% (b)
Propyl-p-hydroxy benzoate	2.30% (a) 1.45% (b)	1.44% (a) 4.15% (b)
Butyl-p-hydroxy benzoate	10.40% (a) 33.20% (b)	15.92% (a) 23.65% (b)

(a) working solution of standard; (b) sample soluton.

The reproducibility of injection for each component was assessed by injecting, in two different days, six replicates of the working solution of standard and a sample of gel. In table 3 are shown the coefficients of variation (C.V.) for each component calculated on area values.

TABLE 4
Accuracy and Precison of Method for Ketoprofen and Parabens

	Accuracy (n=45)	Confidence limits (p = 0.05)	Precison (as C.V.)
Ketoprofen	99.73 %	± 0.24 %	0.48 %
Methyl-p-hydroxy benzoate	99.17 %	± 0.30 %	1.02 %
Ethyl-p-hydroxy benzoate	99.41 %	± 0.20 %	0.68 %
Propyl-p-hydroxy benzoate	99.89 %	± 0.34 %	1.15 %
Butyl-p-hydroxy benzoate	98.96 %	± 2.53 %	8.50 %

The day-to-day precision was determined by conduting the analysis on three different days with three different operators. Accuracy was evaluated by analyzing the prepared samples five times starting from a gel containing known amounts of Ketoprofen and parabens The values found were compared with the actual amounts of the analytes and the values of accuracy and precision of the method, evaluated on 45 analyses, are shown in table 4.

Statistical study (ANOVA) shows that differences between the results of the three different operators and between the results obtained on the three different days are not significant ($p > 0.05$).
The method is sufficiently rapid to be applied to routine analysis, however, due to the specificity, precision and accuracy of the developed HPLC method, it should find application to stability studies of such Ketoprofen and parabens containing gels.

REFERENCES

1) R.N.Brodgen,T.M.Speight and G.S.Avery, *Drugs*, 8 168 1974

2) R.Graham and H.C.Burry, *Scand.J.Rheumatol.*, 14 133 1976

3) E.C.Huskisson (Editor), *Anti-Rheumatic Drugs*, Praeger, New York, 1983

4) A.Kraus and G.Ibanez de Kasep, *Rev.Invest.Clin.*, 37 153 1985

5) M.Canuti, P.Bechelli, G.Cerchiai, *Giornale It.Ric. Clin. Terap.*, 303,suppl. 7, 81 1981

6) G.Vitali, *Clinica terapeutica*, 94 257 1980

7) P.Bechelli, D.Attina and V.Pieri, *Policlinico Sez. Medica*, 3-8,1980

8) P.Pietta, E.Manera and P.Ceva, *J.Chromatogr.*, 390 454 1987

9) P.Pietta, E.Manera and P.Ceva, *J.Chromatogr.*, 387 525 1987