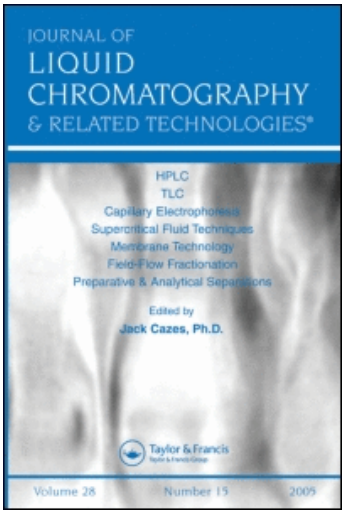

Journal of Liquid Chromatography & Related Technologies

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

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To cite this Article Karnes, H. Thomas , Rajasekharaiah, Kusuma , Small, Ralph E. and Farthing, Don(1988) 'Automated Solid Phase Extraction and Hplc Analysis of Ibuprofen in Plasma', Journal of Liquid Chromatography & Related Technologies, 11: 2, 489 — 499
To link to this Article: DOI: 10.1080/01483918809349954
URL: http://dx.doi.org/10.1080/01483918809349954

JOURNAL OF LIQUID CHROMATOGRAPHY, 11(2), 489-499 (1988)

AUTOMATED SOLID PHASE EXTRACTION AND HPLC ANALYSIS OF IBUPROFEN IN PLASMA

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ABSTRACT

Ibuprofen is a non-steroidal anti-inflammatory drug, widely used in arthritis and other disorders. We describe a high pressure liquid chromatographic (HPLC) method for the analysis of ibuprofen in plasma, using an automated solid phase extraction technique (the Varian AASP^R). In this method ibuprofen was extracted from 0.5 ml of plasma by application to a C_2 extraction cartridge followed by "on line" elution with the HPLC mobile phase (55% acetonitrile / 45% 0.02 M phosphate buffer; pH 3.0), at a flow rate of 1.5 ml/min. The analytical column was a Nucleosil C_{18} column and the fluorescence detector was set at 253 nm (excitation wavelength) and 300 nm (emission wavelength). Chromatography was complete in less than 10 mins and the limit of detection was 1.3 μg/ml. The method is linear through the range of 1.0 to 100.0 μg/ml with a mean correlation coefficient of 0.9964. Absolute recovery of ibuprofen from the spiked plasma samples ranged from 77.8% to 86.5%. The method was shown to be precise within 11% C.V. and accurate to within 8% over the concentration range studied.

INTRODUCTION

Ibuprofen (Fig. 1) is a non-steroidal anti-inflammatory drug used in the treatment of several forms of arthritis and also in the relief of mild to moderate pain. Several methods have been published for the determination of ibuprofen in plasma. Among them are gas-liquid chromatography (GLC) (1-5) and high performance liquid chromatography (HPLC) (6-11).

The published GLC methods require large sample volumes and derivatization steps are required in several cases. Most of the published HPLC methods employ liquid-extractions prior to chromatography. These extractions generally involve precipitation by the addition of organic solvents followed by filtration or centrifugation to remove the precipitated proteins, acidification of the plasma, extraction with the organic solvent, aspiration of the aqueous layer, evaporation of the organic layer and dissolution of the residue in an appropriate solvent.

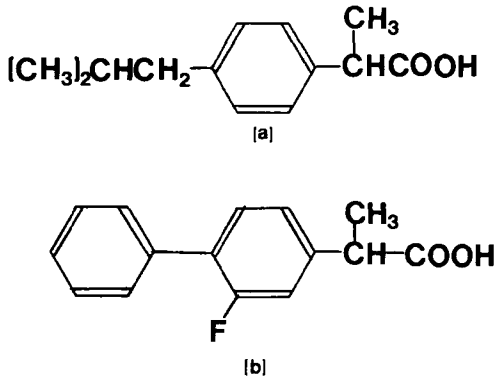

Figure 1 Chemical Structures of a) ibuprofen and b) the internal standard flurbiprofen.

All these processes are time consuming and are prone to the introduction of errors.

Jonkman et al (9) has published a solid phase extraction technique using disposable cartridges, followed by reversed phase HPLC, for ibuprofen. This technique has several intermediate steps however which increase the total analysis time.

Snider et al (8) has developed a sorbent extraction technique for ibuprofen which uses a micro-processor controlled centrifugal system to force the samples through the extraction cartridges which are packed with a macro-reticular resin. This technique is also time consuming because the extract has to be evaporated to dryness and the residue reconstituted in the mobile phase. The retention time for ibuprofen by this method is also extremely long (22 mins).

The Varian AASP^R (advanced automated sample processor) utilizes the principles of solid phase extraction. This system is unlike conventional sorbent extraction in that it is comprised of miniature cartridges converted into unitized molded cassettes. The automated processing is carried out in stages comprised of: 1. extraction cassette processing, 2. pre-injection purge, 3. isolate elution (injection), 4. valve reset and 5. post injection purge. The analyte of interest is isolated in the cassette processing stage which involves conditioning of the catridges with a suitable solvent, addition of the sample or standard to the cartridge reservoir along with the internal standard and passing the above through the cartridges by the application of positive pressure. The sorbent bed is then washed with an appropriate solvent to remove interferences and the processed cassette is loaded onto the AASP. The AASP is connected to the HPLC mobile phase flow through a switching valve.

The AASP contains a programmable purge system which removes air from the lines and washes weakly retained components. This allows clean up of the chromatographic front and enables the detection of early eluting peaks. The purge system can be utilized for pre-column derivatization by substitution of a derivatizing reagent for the wash solution. The purge system can also be programmed to wash all the lines to and from the AASP and prime all these lines with the purge solvent in preparation for the next cassette.

Analyte elution occurs when the injection valve switches the mobile phase through the AASP cassette and then onto the HPLC column. The valve reset time can be programmed to insert and remove the AASP from the mobile phase flow at predetermined intervals, thus allowing potentially interfering late eluting peaks to be trapped on the cartridge.

A powerful advantage of the AASP system is this combination of purge and valve reset which allows the establishment of an " elution window " for the selection of analytes and the removal of interferences. Automation of both extraction and injection by the AASP results in shorter analysis time and consequently reduces the cost per analysis. Accuracy and precision of the analysis is also significantly improved in our experience. The only major drawback with the use of the AASP in the automated mode is that the HPLC mobile phase must be used as the elution solvent. This usually does not pose a problem if the extraction sorbent has a weaker interaction with the analyte than the analytical column packing but limits the use of both stronger eluting solvents and strong extracting sorbent materials.

MATERIALS AND METHODS

Reagents

All solvents were HPLC grade. Acetonitrile, methanol and potassium di-hydrogen phosphate were obtained from Fischer Scientific Company (Fairlawn, N.J.). Ibuprofen was obtained from Sigma Chemical Company (St.Louis, Missouri) and flurbiprofen from The Upjohn Company (Kalamazoo, Michigan).

The ibuprofen stock standard solution (1.12 mg/ml) was prepared in methanol. Dilutions were made from this stock to provide the various concentrations (1.0, 5.0, 10.0, 25.0, 50.0 and 100 μg/ml) of ibuprofen in plasma necessary. Direct injections of standard solutions were analysed for each standard point concentration in recovery studies. Aliquots of spiked plasma solutions were stored frozen to be run as controls in precision studies. The control stock solution (0.916 mg/ml) and the internal standard (flurbiprofen) stock solution (0.5 mg/ml) were prepared in the same manner as the standard stock solution.

Instrumentation and Chromatographic Conditions

A Gilson System III liquid chromatograph equipped with a Varian AASP^R (Advanced Automated Sample Processor) and a Kratos Model 970 fluorescence detector were used. The analytical column was a Nucleosil C_{18} (25 cm x 0.46 cm) 5 μm reversed phase column. The extraction cartridge cassettes were obtained from Analytichem International (Harbor City, California). The AASP and the AASP^R Vacelut system for processing the cassettes were supplied by Varian Instruments (Sugarland, Texas).

Sample injection from the AASP^R onto the analytical column was fully automated and a Gilson Datamaster equi-

pped with an Apple Model II E computer was used for data collection and integration. Valve reset time for the AASP^R was set at 5 mins and the prime purge volume was 75 μl. The excitation wavelength of the fluorescence detector was 253 nm and the emission wavelength was 300 nm. The sensitivity was set at 0.5 m.V.f.s. with a time constant of 6 secs. The mobile phase consisted of 55% acetonitrile and 45% 0.02 M phosphate buffer (pH 3.0). The flow rate was 1.5 ml/min at ambient temperature.

Extraction Procedure

The C_2 extraction cartridges were conditioned by passing 1.0 ml of methanol followed by 1.0 ml of the wash solution (0.5 M phosphoric acid) through the cartridge. One half milliliter of sample or standard was added to the cartridge reservoir, followed by 20 μl of the internal standard (2.5 μg/ml solution of flurbiprofen) and 0.5 ml of the wash solution. This mixture was then passed through the cartridge by the application of positive pressure on the inlet side of the reservoir. The cartridge was then washed with 0.5 ml of the wash solution. The cassette was then loaded onto the AASP for on-line elution of the extracted ibuprofen and internal standard.

RESULTS AND DISCUSSION

A chromatogram of the blank plasma demonstrating the absence of interfering peaks at the retention times of interest is shown in Fig.2. A chromatogram of serum spiked with ibuprofen and the internal standard is shown in Fig.3. The excitation wavelength of 253 nm was adequate

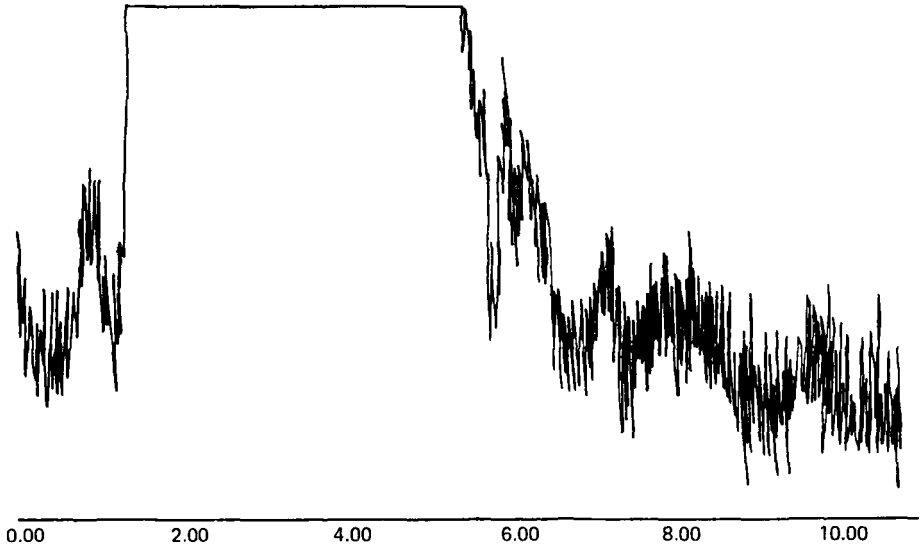

Figure 2 Chromatogram of Blank Serum showing the absence of interfering peaks at the retention times of interest with sensitivity set at 0.18 m.V.f.s.

for the detection of both ibuprofen and the internal standard although the maximum excitation wavelength for ibuprofen is 227 nm. The use of this wavelength with an alternate internal standard would possibly enable the detection of smaller quantities of ibuprofen.

Interferences due to the presence of other drugs administered concomittantly such as diazepam, carbamazepine, theophylline, cimetidine, primidone, caffeine, acetaminophen, propranolol and clonazepam were ruled out after analyzing these drugs in the current system at the concentrations normally present in plasma. In all cases there were no interfering peaks.

The results obtained from absolute recovery studies are given in Table 1. The absolute recoveries obtained

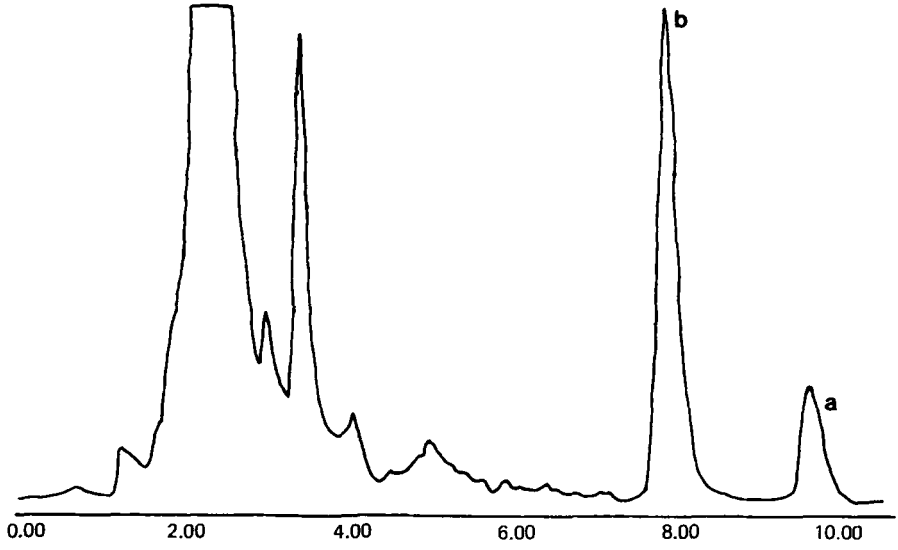

Figure 3 Chromatogram of Serum Spiked with 10 μg/ml of a) ibuprofen (t_r = 9.7 mins) and b) the internal standard (t_r = 8.0 mins) with sensitivity set at 6.92 m.V.f.s.

TABLE 1
Recovery Data

Target Conc. (μg/ml)	Mean Area (extracted)	Mean Area (direct inj.)	Recovery %
100	420347	533030	78.9
50	224155	288208	77.8
25	115235	145553	79.2
10	46502	53778	86.5

TABLE 2
Precision and accuracy

Spiked Conc. (μg/ml)	Mean Assayed Conc. (n = 6)	Coefficient of Variation	Relative Error %
75	72.3	6.0	3.6
40	38.7	4.4	3.3
15	16.2	11.0	8.0

from the lot of cartridges used was consistent and adequate.

Calibration curves obtained from standards containing 1.0 to 100.0 μg/ml of ibuprofen are linear and demonstrated correlation coefficients (n=18) between 0.996-0.997 with a mean of 0.9964. The mean regression equation is $y = 0.025x - 0.003$, where x and y are peak areas and ibuprofen concentrations respectively. The limit of detection of this method is 1.3 μg/ml (three times the standard deviation of the lowest back calculated standard point) which is adequate for the routine analysis of plasma samples.

Precision and accuracy data are given in Table 2. This data is the mean of six determinations at each spiked concentration. The coefficient of variation for the assay ranges from 6.0 to 11.0. Relative error is between 3.6% and 8.0% at the lower concentrations.

Conclusion

A simple, specific and an automated solid phase extraction followed by HPLC for the determination of ibuprofen has been developed. The extraction time has been reduced to approximately 2-5 mins as compared to traditional liquid-liquid extraction processes which commonly require 1-2 hrs. The use of fluorescence detection reduced or eliminated the background interferences

thereby increasing selectivity. The assay is suitable for pharmacokinetic studies of ibuprofen in adults and in children because of the small sample volumes required.

ACKNOWLEDGEMENTS

The authors would like to acknowledge the Varian Instrument group for use of the AASP which was employed in this study and we would also like to thank Dr. John Wood for his advice.

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