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Journal of Pharmaceutical and Biomedical Analysis
25 (2001) 871–879

JOURNAL OF
PHARMACEUTICAL
AND BIOMEDICAL
ANALYSIS

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The use of hydrophilic lipophilic balanced (HLB) copolymer SPE cartridges for the extraction of diclofenac from small volume paediatric plasma samples

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Received 25 October 2000; received in revised form 13 December 2000; accepted 29 December 2000

Abstract

The extraction of diclofenac from spiked aqueous and plasma samples by liquid–liquid extraction (LLE) and solid phase extraction (SPE) methods is compared. The SPE methodology utilised a hydrophilic lipophilic balanced (HLB) copolymer as the extraction phase. Using a literature HPLC method, a calibration curve for diclofenac was constructed in the range 1.0–50.0 μg/ml. Diclofenac spiked samples (aqueous and plasma) were extracted by LLE and SPE methodologies. The SPE resulted in higher extraction efficiencies (mean 94.9%) than the LLE (mean 78.9%) with %R.S.D.s similar in both methods (3.2 vs. 2.1%, respectively). The SPE method was suitable for the extraction of diclofenac from small volume paediatric plasma samples.

Keywords: Diclofenac; Solid phase extraction (SPE); Paediatric; Plasma; HPLC

1. Introduction

There is, at present, great concern regarding the unlicensed and off label use of drugs in paediatric patients [1–3]. One reason for the lack of paediatric licenses is the ethical problems associated with conducting conventional clinical trials in children. We have recently initiated a project which aims to provide an evidence base for optimising the dosage of drugs in hospitalised paediatric patients. This work involves the utilisation of population pharmacokinetics [4] in place of conventional pharmacokinetic protocols. This entails the collection of blood samples from paediatric patients who have been prescribed the study drugs for clinical reasons. Sampling requires the collection of small volume blood samples at the same time as routine samples are collected for the clinical biochemistry laboratory. The population pharmacokinetic protocol involves infrequent (sparse)

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0731-7085/01/$ - see front matter
PII: S0731-7085(01)00367-3

sampling from a large population. Drug level determination is used in conjunction with clinical data to evaluate the pharmacokinetics, efficacy and safety of the drugs under investigation.

Diclofenac (Fig. 1(i)) is one such unlicensed drug (non-steroidal anti-inflammatory) used in the paediatric population as a post-operative analgesic. There are no published articles specifically concerned with the determination of this drug in small volume paediatric samples, although, a number of methods have described its determination in biological matrices from adults [5–9]. Liquid–liquid and silica based solid phase extraction (SPE) techniques have been described for the isolation of diclofenac from biological matrices. The liquid–liquid extraction procedures described are time consuming [5–7] and the SPE methods involving conventional silica based packings [8,9] have been reported to display widely varying mean recoveries of 70% [8] and 101% [9] for similar C_{18} packing materials. These difficulties prompted us to investigate the usefulness of one of the newer polymer based SPE systems (a hydrophilic lipophilic balanced [HLB] copolymer)

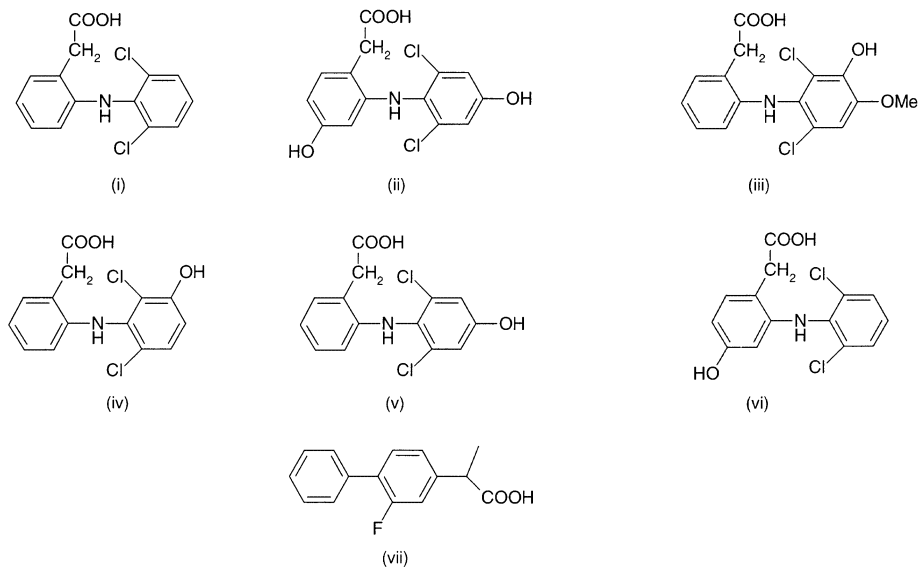

Fig. 1. The structures of diclofenac (i); the metabolites of diclofenac (4′,5-dihydroxydiclofenac (ii); 3′-hydroxy-4′-methoxydiclofenac (iii); 3′-hydroxydiclofenac (iv); 4′-hydroxydiclofenac (v); and 5-hydroxydiclofenac (vi)); and the internal standard flurbiprofen (vii).

Table 1
Mean recovery data and %R.S.D. for liquid–liquid (LLE) and solid phase extraction (SPE) of diclofenac from spiked aqueous and plasma samples

Extraction method	Sample	Diclofenac conc. (μg/ml)	Mean recovery %[a]	% R.S.D.
LLE	H_2O	6–8	79.7	4.7
LLE	H_2O	0.4–0.6	74.1	4.1
LLE	plasma	6–8	85.1	0.2
LLE	plasma	0.4–0.6	76.6	3.6
SPE	H_2O	6–8	98.1	4.0
SPE	H_2O	0.4–0.6	93.0	1.5
SPE	plasma	6–8	98.8	0.3
SPE	plasma	0.4–0.6	89.8	2.7

[a] $n = 5$.

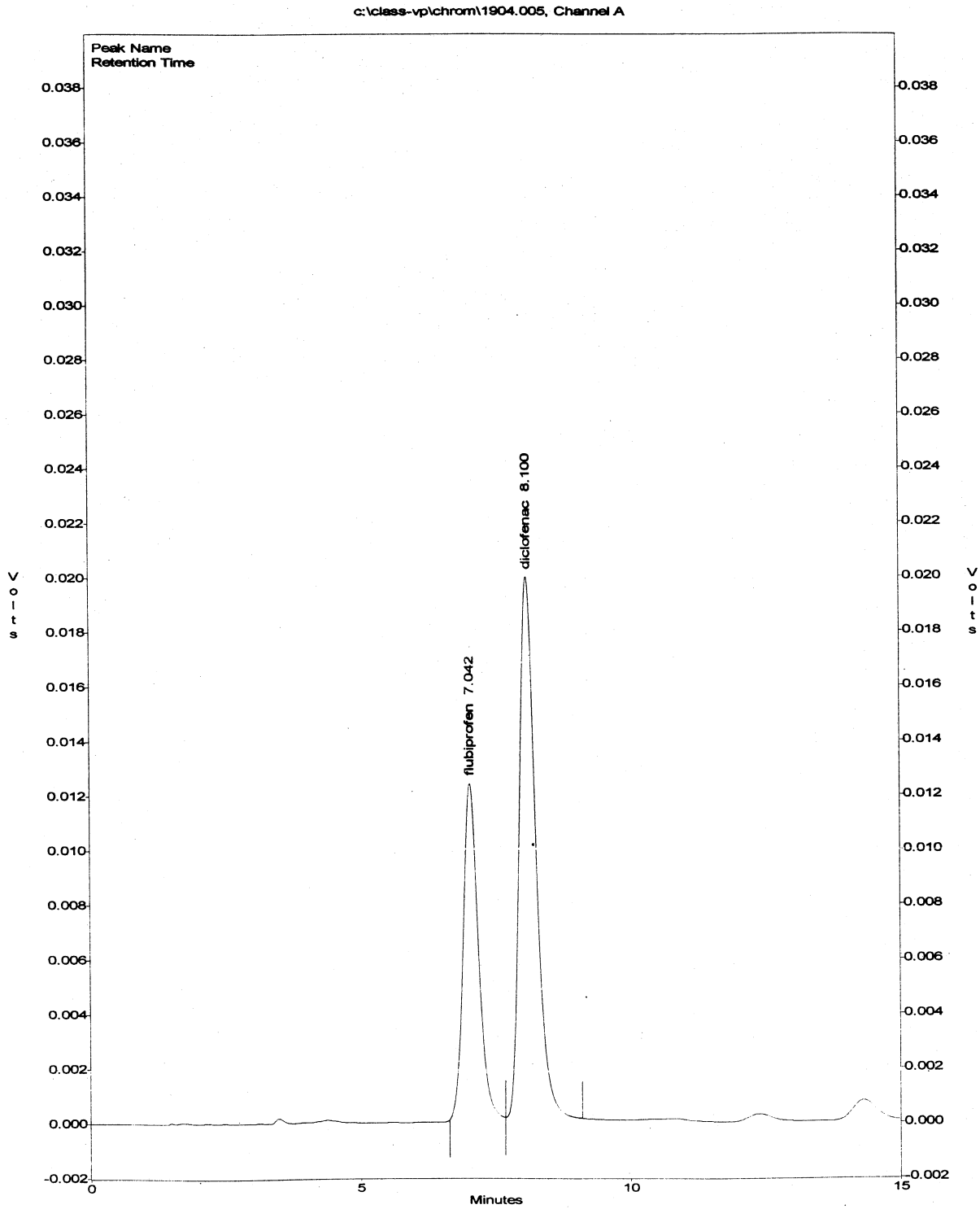

Fig. 2. HPLC chromatogram of a standard sample of diclofenac (25 μg/ml) and the internal standard flurbiprofen (25 μg/ml).

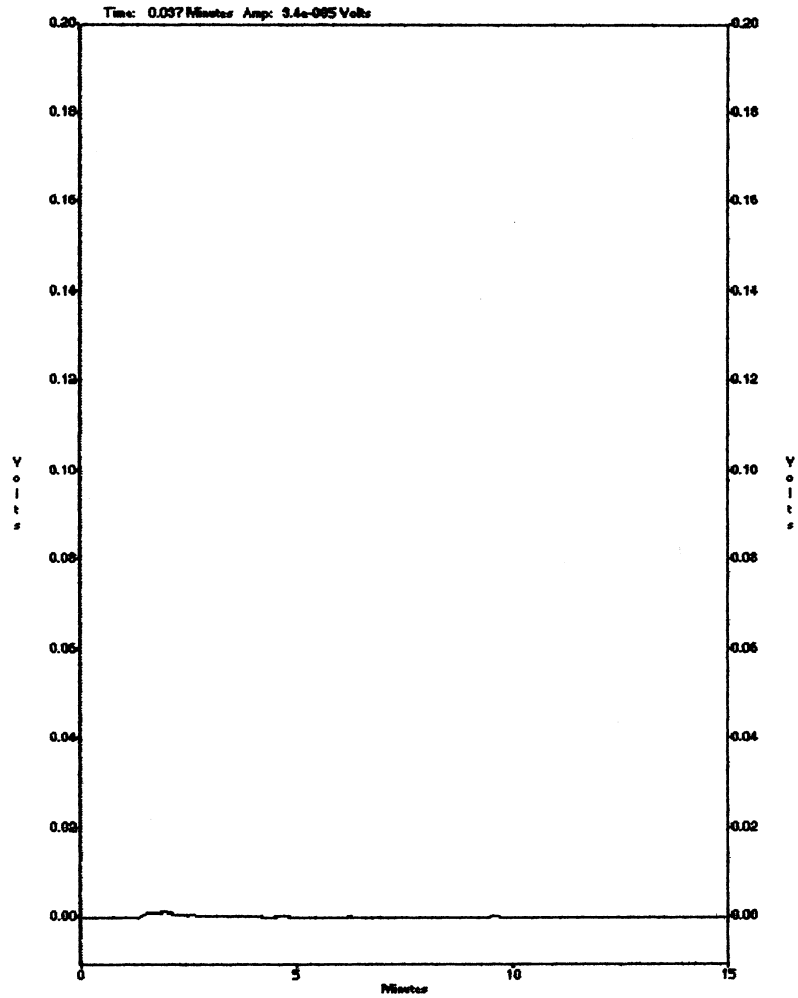

Fig. 3. HPLC chromatogram of a blank aqueous sample following SPE.

for the extraction of diclofenac from small volume (0.5 ml) paediatric plasma samples.

2. Experimental

2.1. Materials

Diclofenac sodium and flurbiprofen (IS) were purchased from Sigma (Poole, England). Methanol, propan-2-ol (IPA) and hexane were HPLC grade (Lab Scan Analytical Services, Dublin). Orthophosphoric acid AR was obtained from BDH (Poole, England). All water was processed using a Millipore-Q Reagent System (Waters, England). Filtration of HPLC mobile phases was performed using Gelman FP-450 Filters (Pall Gelman Sciences, Northampton, England). Diclofenac metabolites were kindly donated by Novartis, Basel, Switzerland (Fig. 1). The Northern Ireland Blood Transfusion Service provided screened whole blood from which plasma was obtained. The plasma was stored at -20°C until required.

2.2. Instrumentation

The HPLC system included a Shimadzu SCL-10AVP system controller, Shimadzu SIL-10ADVP autoinjector, Shimadzu LC-10ATVP pump, Shimadzu FCV-10ALVP solvent mixer, Shimadzu DGU-14A degasser and a Jones Chromatography model 7990 column heater. The column was a Waters Spherisorb S5 ODS1 (4.6 × 125 mm) fitted with a Waters Spherisorb S5 ODS1 (4.6 × 10 mm) guard column. The mobile phase consisted of MeOH/Water (adjusted to pH 3.3 with H_3PO_4) (63:37) pumped at 1 ml/min. UV detection was employed at 280 nm and the column temperature was maintained at 30°C. A 20 μl sample injection volume was used.

2.3. HPLC determination of diclofenac

Using the system outlined above a diclofenac calibration curve was constructed in the range 1–50 μg/ml following the published method of Blagbrough et al. [5] using flurbiprofen as the internal standard. Peak area ratios were plotted against diclofenac concentration in the construction of the calibration curve. The retention times of (i); the metabolites of diclofenac (4′,5-dihydroxydiclofenac, (ii); 3′-hydroxy-4′-methoxydiclofenac;

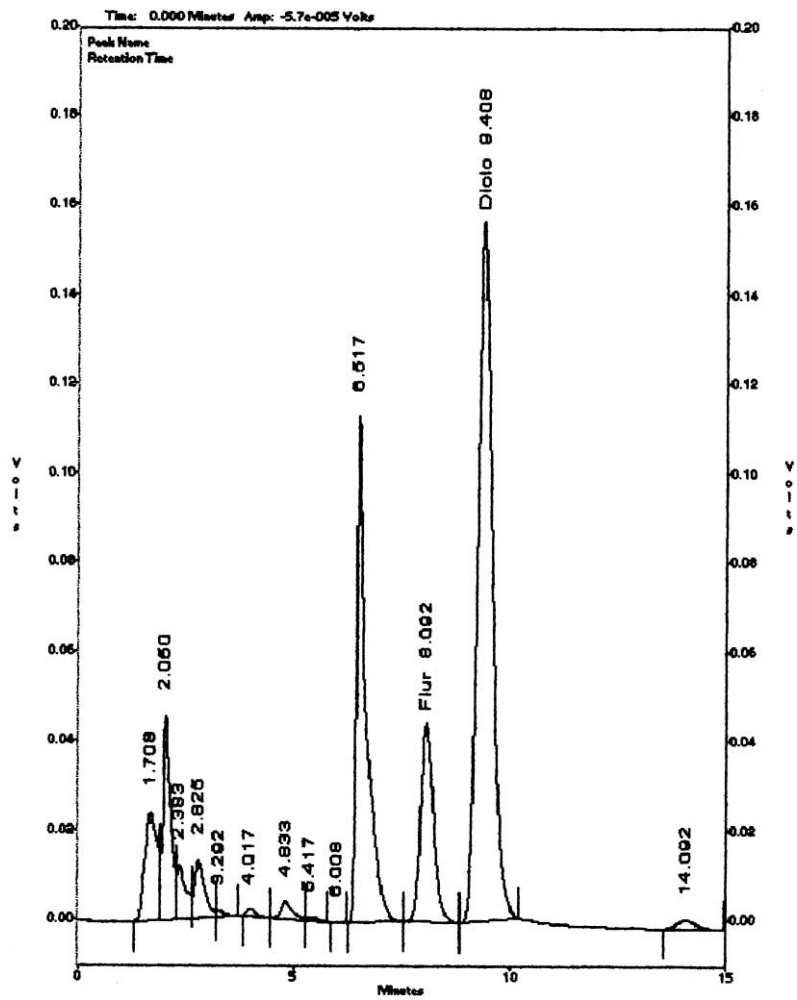

Fig. 4. HPLC chromatogram of an extracted paediatric plasma sample following SPE.

(iii); 3′-hydroxydiclofenac, (iv); 4′-hydroxydiclofenac, (v); and 5-hydroxydiclofenac; (vi); Fig. 1) were determined in order to check on the possible interference of these metabolites with the peaks due to diclofenac or the internal standard.

2.4. Extraction

In both extractions described below the initial concentrations of diclofenac in the spiked samples were selected so as to take account of concentration enrichment when the sample was finally redissolved in 150 μl of mobile phase prior to the final analysis.

2.5. Liquid–liquid extraction

Liquid–liquid extraction was carried out according to the method of Li et al. (1995). The sample (aqueous or plasma) (0.5 ml), spiked with diclofenac, was acidified using 0.6 ml of 1 M H_3PO_4 and the solution vortexed for 10 s. 5 ml of the extraction solvent (Hexane:IPA 95/5 (v/v)) was added and the mixture vortexed for 1 min followed by centrifugation for 10 min at $3000 \times g$. Following centrifugation, 4 ml of organic layer was transferred to a clean glass centrifuge tube and evaporated to dryness at 40°C under a stream of nitrogen. The residue was reconstituted in 150 μl of mobile phase containing the internal standard (25 μg/ml).

3. Solid phase extraction

The solid phase extraction procedure utilised HLB solid phase extraction cartridges (1 cc; 30 mg: Oasis, Waters, England) in conjunction with a Waters extraction manifold system (20 position manifold with a 13×75 mm test tube rack). The vacuum pressure on the manifold was maintained at ≤ 5 inch (12.7 mmHg) throughout the duration of the SPE protocol. The cartridges were conditioned using 1 ml of methanol and 1 ml of water. Plasma (0.5 ml) spiked with diclofenac was acidified using 0.6 ml of 1 M H_3PO_4 and the solution loaded onto the extraction cartridge. A wash step was performed using 1 ml of 5% aqueous methanol. The waste solvents were discarded and the rack filled with collection tubes (disposable Pyrex borosilicate glass culture tubes, Corning, New York) and the sample eluted using 1 ml of methanol. The sample was evaporated to dryness at 40°C under a stream of nitrogen. The residue was reconstituted in 150 μl of mobile phase containing the internal standard (25 μg/ml). Additionally, solid phase extraction of aqueous samples of diclofenac was conducted following the above methodology to determine optimum SPE recovery and to ensure no interference from the cartridge materials.

4. Results and discussion

The HPLC method used in this study was based on that described by Blagbrough et al. [5]. Using this literature method diclofenac was found to elute at approximately 9.5 min, whilst the internal standard, flurbiprofen, eluted at approximately 8.1 min with the two compounds baseline resolved (Fig. 2). Calibration curves (using peak area ratios) were constructed using standard aqueous solutions of diclofenac and the internal standard flurbiprofen (Fig. 1; vii). These curves were linear over the range 1–50 μg/ml, Eq. (1) is an example of a typical curve (with S.D. of slope and intercept included in brackets). The limit of quantification was 1 μg/ml and the limit of detection was 42 ng/ml. Initial studies using identical guard and analytical columns but with an alternate HPLC system had resulted in the generation of equivalent calibration curves.

$$y = 0.0670(0.0004) \times -0.0070(0.0099)$$
$$r^2 = 0.9991 \quad (1)$$

The concentrations of diclofenac following both LLE and SPE were determined using the calibration curve. The results of both extractions are detailed in Table 1. Liquid–liquid extraction of an aqueous sample of diclofenac gave a chromatogram essentially identical to that shown in Fig. 2. The chromatogram following liquid–liquid extraction of a blank plasma sample showed no interference from endogenous components in the

region where diclofenac and flurbiprofen eluted. The chromatogram of a solid phase extraction of an aqueous sample containing no diclofenac and reconstituted using mobile phase containing no internal standard showed that there were no interferences due to polymeric components being washed from the cartridge during the extraction procedure Fig. 3. Fig. 4 shows the chromatogram resulting from an extracted paediatric plasma sample that had been subjected to SPE. The chromatographic data demonstrated clearly that both extraction procedures and the chromatographic methodology are suitable for the extraction and quantification of diclofenac.

Table 1 shows comparative data for the two extraction procedures using both aqueous and plasma samples spiked with diclofenac. These data demonstrate that both methods yield high recoveries suitable for the extraction of diclofenac. The liquid–liquid extraction gave recoveries (74.1–85.1%), which were lower than those reported by the original authors (91.3–93.2%). Earlier attempts, in our laboratories, to follow literature reported extraction procedures for diclofenac had also resulted in lower than reported recoveries. The recoveries obtained in the SPE extractions were significantly higher (89.8–98.8%) than those obtained following LLE with the %R.S.D. similar in both methods. Fig. 5 shows the overlaid chromatograms of diclofenac, flurbiprofen (IS) and the five metabolites run under identical conditions; this figure shows that there is no interference between peaks due to the metabolites and the peaks for diclofenac and the internal standard.

Liquid–liquid extraction was, for many years, the major procedure for the isolation of drug substances from biological matrices prior to the determination of their concentration. LLE procedures, however, suffer from a number of disadvantages including large solvent consumption, time/labour intensive methodologies, difficulties involved in achieving optimum conditions required to obtain high analyte distribution coefficients and the problems associated with samples which form emulsions during extraction. More recently, SPE utilising materials developed for High Performance Liquid Chromatography (HPLC), has become an accepted technique in drug analyses. The advantages of SPE have been extensively reported yet despite this a number of difficulties have been observed including sorbent drying (resulting in cracking of the packing material), polar analyte breakthrough and strong retention of basic compounds due to interaction with silanol groups. Recently, a number of newer packing materials have been introduced for SPE that are polymer based in comparison to conventional SPE phases which are primarily silica based. These newer materials are reported to be superior to the conventional phases in terms of sorbent drying, polar analyte breakthrough and basic compound retention. The investigation described above involved one of these newer SPE phases that is based on a hydrophilic lipophilic balanced copolymer derived from vinylpyrrolidone and divinylbenzene. The vinylpyrrolidone monomer contributes the hydrophilic characteristics providing wetting properties, which allows sorbent drying without recovery problems, whereas the divinylbenzene monomer provides the reverse phase characteristics for analyte retention. The copolymer sorbent can be used to extract compounds over a wide pH range (0–14) compared with the silica based packings which have a narrower operational pH window (2–8).

In this study, the polymeric phase produced excellent results in terms of high recovery and low %R.S.D. Such results were independent of vacuum manifold use, as preliminary investigations in the present study involving the use of a peristaltic pump to draw solutions through the cartridges resulted in high recoveries and low %R.S.D. similar to those reported above. The hydrophilic properties of the cartridges used in this study were briefly examined by purposely allowing a number of cartridges to dry out on the manifold. This did not result in any significant variation in the percentage recovery nor did it result in changes to the endogenous substance profile observed. This would indicate, at least in this study, that the need to carefully monitor cartridge drying, as is the case when using conventional silica based SPE, is not necessary with these polymeric based SPE cartridges. The procedure involved in this SPE methodology is considerably

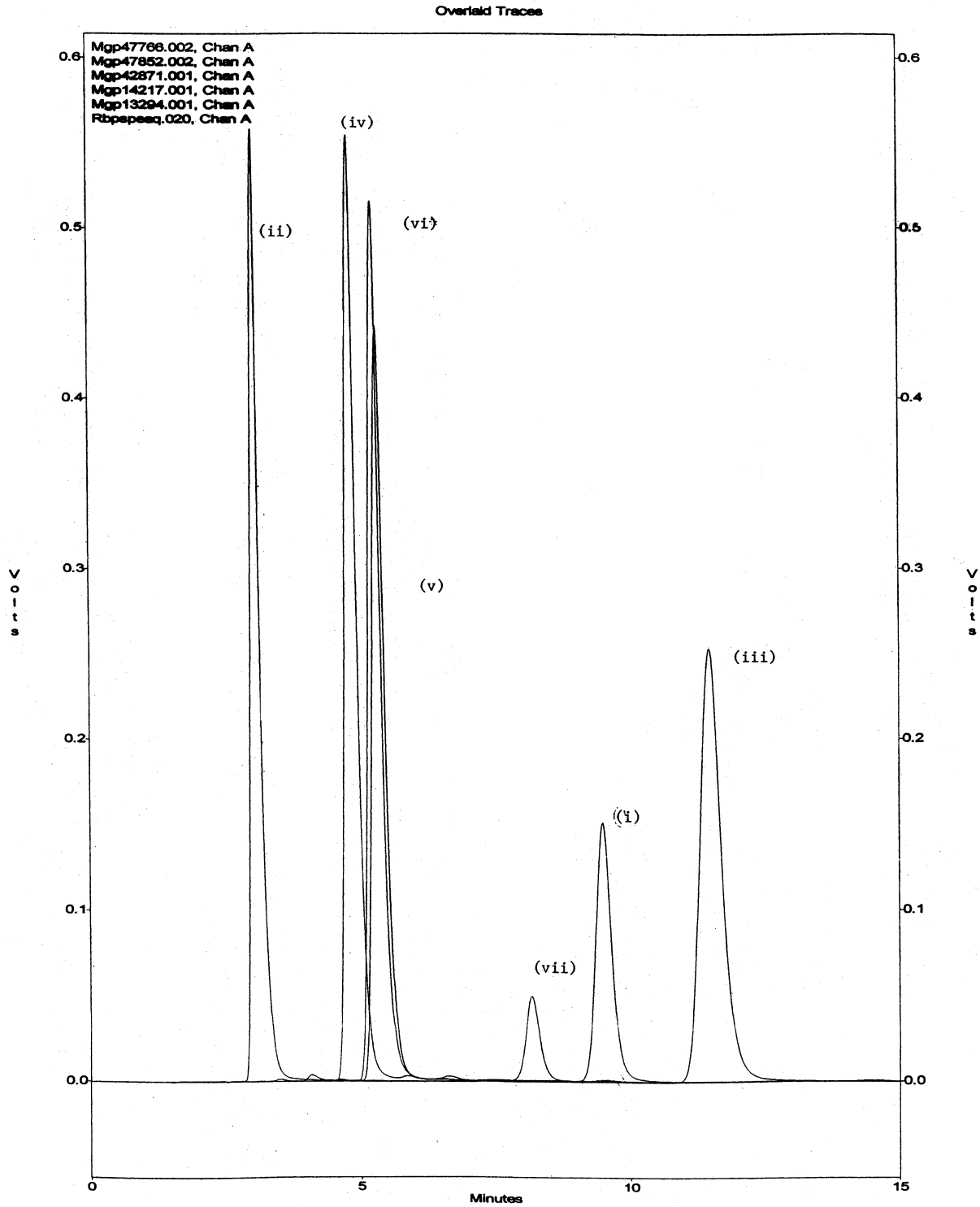

Fig. 5. Overlaid HPLC chromatograms of diclofenac (i); the metabolites of diclofenac (4′,5-dihydroxydiclofenac (ii); 3′-hydroxy-4′-methoxydiclofenac (iii); 3′-hydroxydiclofenac (iv); 4′-hydroxydiclofenac (v); and 5-hydroxydiclofenac (vi)) and the internal standard flurbiprofen (vii). (Compounds (ii–vi) conc. 100 μg/ml).

faster than the conventional LLE extraction especially when the extraction manifold is used thereby allowing twenty extractions simultaneously.

5. Conclusions

The use of the HLB copolymer extraction cartridges for the extraction of diclofenac from plasma results in high analyte recoveries with low %R.S.D. The extraction of diclofenac from plasma using these cartridges is simple, fast and does not appear to be affected by sorbent drying. The methodology is suitable for the extraction of diclofenac from small volume paediatric plasma samples.

Acknowledgements

The authors wish to acknowledge the support of Action Research through grant S/P/3273.

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