ELSEVIER

Journal of Pharmaceutical and Biomedical Analysis
17 (1998) 679–687

JOURNAL OF
PHARMACEUTICAL
AND BIOMEDICAL
ANALYSIS

Solid-phase extraction and liquid chromatographic quantitation of the antiarrhythmic drug L-768673 in a microemulsion formulation

R.R. Kenney *, R.J. Forsyth, H. Jahansouz

Pharmaceutical Research and Development Department, Merck Research Laboratories, West Point, PA 19486, USA

Received 25 June 1997; accepted 28 October 1997

Abstract

A sensitive and specific method based on solid-phase extraction and reverse-phase liquid chromatography was developed and validated for the quantitation of L-768673 in a microemulsion formulation. Following a water wash, the drug was eluted from the extraction column with acetonitrile and was analyzed on a reverse-phase C18 column with UV detection at 245 nm. The mobile phase consisted of acetonitrile–0.2% trifluoroacetic acid, 0.1% triethylamine (53:47 v/v). The retention time of L-768673 was approximately 28 min with a flow rate of 1.5 ml min^{-1}.

Keywords: L-768673; Microemulsion assay; High performance liquid chromatography; HPLC; Solid-phase extraction

1. Introduction

L-768673 (Fig. 1) is a new class III antiarrhythmic drug. The crystalline drug is practically insoluble in water. Therefore, it is being developed in a microemulsion formulation. Concentrations of the drug in the microemulsion were 0.1, 0.05 and 0.0125 mg ml^{-1}.

The concentration of the drug at 0.1 mg ml^{-1} can be determined in the microemulsion by dissolving the formulation in dimethylacetamide (DMA) and assaying by high-performance liquid chromatography (HPLC). Sensitivity for this and other direct preparation methods [1,2] is too limited for detection of low drug levels as well as potential impurities and degradates at 0.1% of the active concentration. For the 0.0125 mg ml^{-1} formulation, the limit of quantitation for potential degradates is 10% of the assay concentration. Spectrophotometric assay is not feasible due to interference from the components of the microemulsion which overwhelm absorbance of the drug at lower concentrations.

The objective of this development project was to achieve a highly sensitive, stability indicating method for analysing L-768673 in the microemulsion formulation, particularly at low drug concentrations. A more sensitive method was developed using solid-phase extraction to concentrate L-

* Corresponding author. Fax: +1 215 6522835; e-mail: robert_kenney@merck.com

0731-7085/98/$19.00
PII S0731-7085(97)00253-7

768673 on increased sensitivity and allow selective recovery of the drug from the emulsion matrix. The sample was then assayed using HPLC with UV detection.

2. Experimental

2.1. Reagents and materials

All chemicals were of analytical grade and were purchased from the following suppliers: Optima grade acetonitrile, Reagent grade *N*,*N*-dimethylacetamide, HPLC grade triethylamine and Certified grade trifluoroacetic acid from Fisher Scientific (Fair Lawn, NJ). Solid-phase extraction columns: Bond-Elut® from Varian (Harbor City, CA), Sep-Pak® from Waters (Milford, MA), Bakerbond® from J.T. Baker (Philipsburg, NJ). Vacuum manifold from Supelco (Bellefonte, PA). HPLC columns: Inertsil® from MetaChem (Torrance, CA), Customsil® from Phenomenex (Torrance, CA), Partisil® from Whatman (Clifton, NJ), Hypersil® from Keystone Scientific (Bellefonte, PA).

2.2. High-performance liquid chromatography

HPLC separations were performed on a Hewlett-Packard 1100 liquid chromatograph equipped with a Hewlett-Packard autosampler, a four solvent proportioning valve solvent delivery system, and a thermostated column compartment. The mobile phase was acetonitrile-0.2% trifluoroacetic acid, 0.1% triethylamine (53:47 v/v) delivered at 1.5 ml min^{-1}. Measurements were made with a 50 or 100 μl injection volume for the 0.1 or 0.01 mg ml^{-1} assay concentrations, respectively, at ambient temperature using a Hewlett-Packard diode-array detector set at 245 nm. Data was processed using Fisons Multichrom® chromatographic software on a Digital VAX computer. Analyses were carried out isocratically on a Hypersil® BDS C18 (250 × 4.6 mm i.d.) 5 μm particle size column. Under these analysis conditions, the elution time for L-768673 was approximately 28 min.

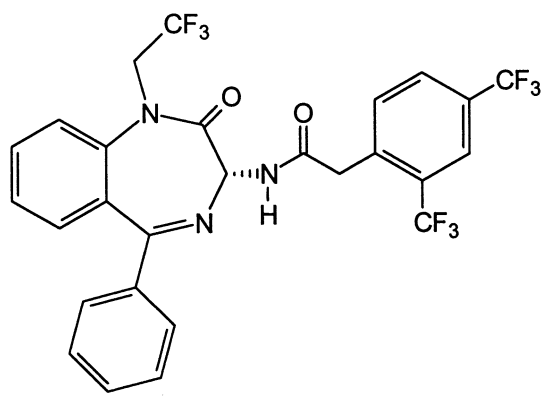
A validated gradient HPLC method using *N*,*N*-dimethylacetamide (DMA) as the diluent was used as an equivalency test. The microemulsion sample (0.1 mg ml^{-1}) was dissolved in DMA and injected onto a Whatman Partisil® HPLC column (250 × 4.6 mm) 5 μm particle size. The gradient mobile phase was A-acetonitrile B-0.1% H_3PO_4. The linear gradient profile was: 0–25 min, A-100% B-0%; 26–50 min, A-43% B-57%; 51–60 min, A-100% B-0%. The flow rate was 1.0 ml min^{-1}, detection was by UV at 245 nm, with an injection volume of 50 μl. The active peak eluted at approximately 41 min in this system.

2.3. Standard solution preparation

Approximately 20 mg of L-768673 was weighed accurately into a 200 ml volumetric flask, dissolved and diluted to volume with acetonitrile:water (80:20 v/v) (0.1 mg ml^{-1} standard). If necessary 5.0 ml of the 0.1 mg ml^{-1} standard was diluted to 50 ml with acetonitrile:water (80:20 v/v) (0.01 mg ml^{-1} standard).

2.4. Treatment of emulsion samples

The vacuum manifold was set up according to the manufacturer's instructions and a 3 cc solid



$C_{27}H_{17}N_3O_2F_9$

Fig. 1. Structure of L-768673.

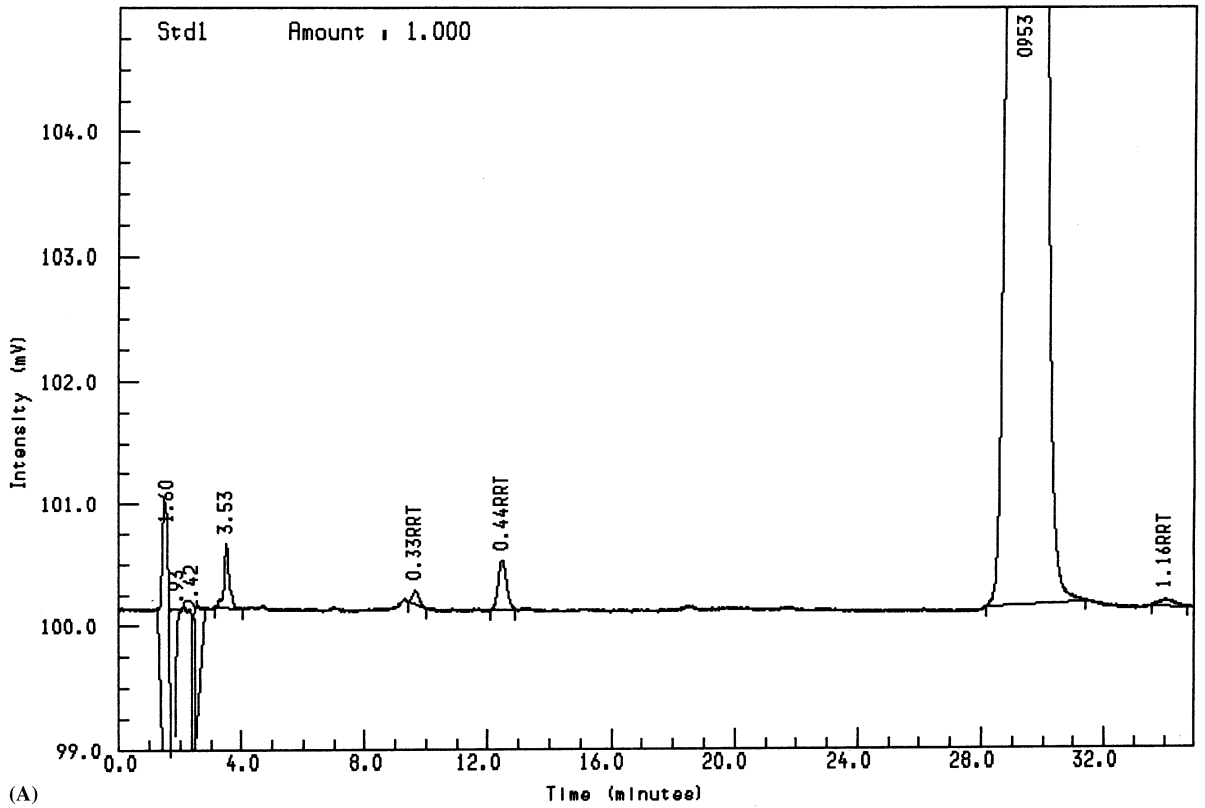


Fig. 2. (A) HPLC chromatogram of L-768 673 bulk material; (B) HPLC chromatogram of placebo microemulsion at 0.1 mg ml^{-1} level; (C) HPLC chromatogram of placebo microemulsion at 0.0125 mg ml^{-1} level; (D) HPLC chromatogram of microemulsion at 0.1 mg ml^{-1} level; (E) HPLC chromatogram of microemulsion at 0.0125 mg ml^{-1} level.

phase extraction column containing 500 mg of C18 packing was installed. The column was washed with approximately 6 ml of acetonitrile, followed by 6 ml of HPLC grade water. The column was charged with 1 ml of the microemulsion and approximately 2 ml of water. The microemulsion–water mixture was allowed to pass through the column drop by drop. This was repeated four more times for a total of five 1 ml charges of the microemulsion and 10 ml of water. The column was washed with water until a clear effluent was obtained (approximately 3–6 ml). Vacuum was applied until all water was removed from the column packing. A volume of 4.5 ml of acetonitrile was added to the column and was slowly collected drop by drop into a 5 ml volumetric flask. The flask was allowed to come to room temperature, where it was brought to volume with water. Duplicate injections of the standard and sample solutions were made into the HPLC system.

3. Discussion

Several analytical methods reported in the literature [3–5] using solid-phase extraction for microemulsion sample preparation, along with an HPLC method using dissolution in DMA, were evaluated for the analysis of L-768673. None of these procedures provided satisfactory results in terms of selectivity, sensitivity and reproducibility. This necessitated the development of an improved, sensitive analytical method for L-768673 in microemulsion which would also demonstrate good extraction column-to-column reproducibility.

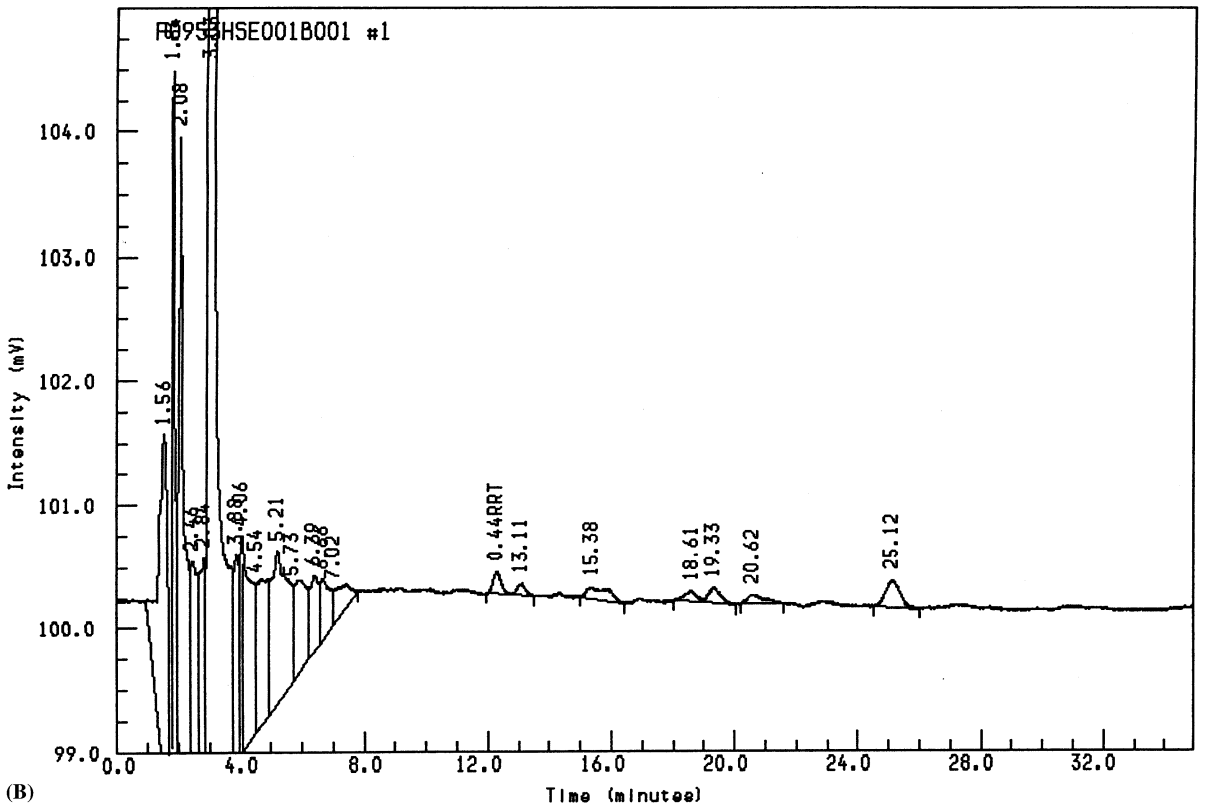


Fig. 2. (*Continued*)

3.1. Selection of sorbent for solid-phase extraction

L-768673 is a neutral compound with a secondary amine group as well as several aromatic ring structures (Fig. 1). The compound is practically insoluble in water. A reverse-phase separation using a non-polar solvent was considered as the primary mechanism for solid-phase extraction. Among the commercially available non-polar sorbents, octyl (C8) and octadecyl (C18) were evaluated for L-768673 in terms of adsorption and desorption.

The components of the microemulsion exhibit a range of polarities. The strategy was to wash off the polar components of the microemulsion using a strong wash solvent, whilst the non-polar L-768673 was retained on the extraction column. An elution solvent was needed which is just strong enough to elute the L-768673 but would leave the less polar microemulsion components on the column.

The retention characteristics of the drug were first evaluated by HPLC. The C18 packing exhibited greater affinity than the C8 packing because of the greater interactions between the C18 and the non-polar drug. The increased affinity for the C18 packing allowed the use of a stronger wash solvent which would be more effective in removing the polar microemulsion components from the matrix.

The amount of packing material was also critical. A solid-phase column with 300 mg of C18 packing was evaluated. Recoveries were not quantitative, indicating that the column was overloaded and that L-768673 was washing through. A 500 mg column provided excellent recoveries and was employed for this method.

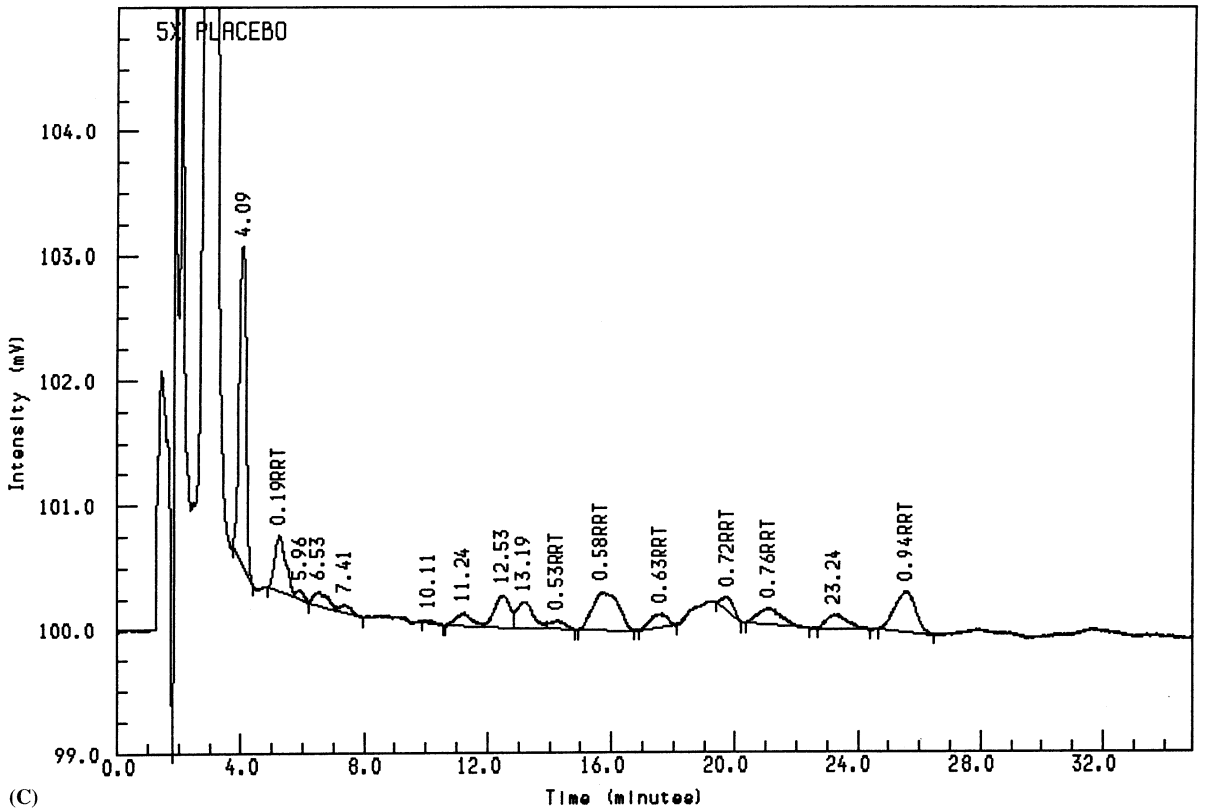


Fig. 2. (*Continued*)

3.2. Optimization of wash and eluting solvent

The preliminary HPLC work demonstrated the affinity of L-768673 for the C18 packing. This work also showed that the greater the percentage of aqueous mobile phase composition, the greater the retention on the column. Water was tested as the wash solvent for the formulation. Water increased the affinity of L-768673 for the C18 packing. The low solubility of the compound also aided in its retention on the column. Diluting the microemulsion with water on the head of the column increased the surface area of the microemulsion and prevented the sample from overloading the extraction column. The extraction column was washed until the effluent became clear which took approximately 5 ml of water.

Mixtures of acetonitrile and water were assessed as the eluting solvent. Low results were obtained using several mixtures. Adding higher percentages of acetonitrile to the mixture did not improve recoveries significantly. The limiting step was the problem of restricted elution volume, which was critical for the necessary sensitivity. The option of evaporative concentration was considered but a more straightforward solution was preferred. Pure acetonitrile was evaluated as the eluting solvent. Recoveries were good but produced chromatograms with poor peak shape. It was decided to elute the drug with 4.5 ml of acetonitrile and then bring the 5 ml volumetric flask to volume with water. This solution provided the strong solvent needed to elute L-768673 and the aqueous component to assure good chromatography.

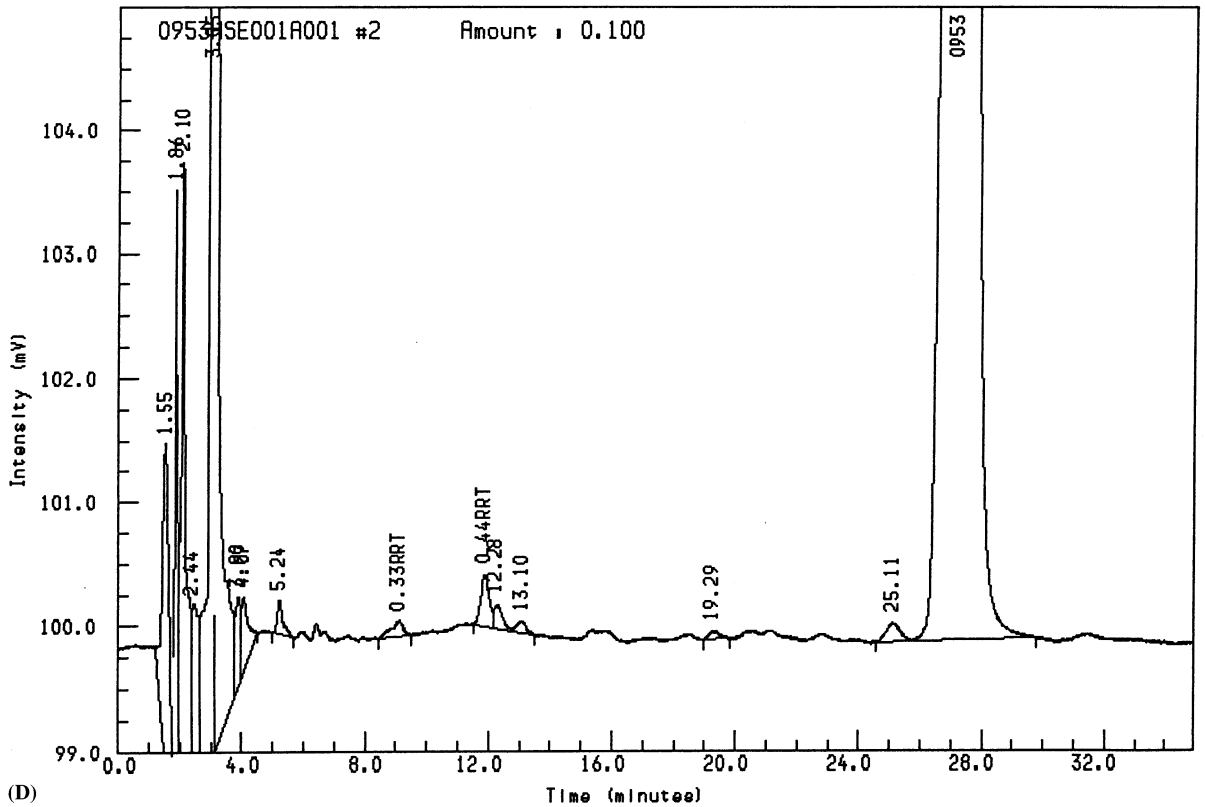


Fig. 2. (*Continued*)

3.3. Selection of HPLC column and mobile phase

Both C8 and C18 columns were evaluated during method development. Both packings provided adequate chromatography but the C18 columns provided better separation between L-768673 and the remaining microemulsion components, which also eluted from the solid-phase extraction column. Several types of C18 columns were assessed for the method. Inertsil® and Customsil® columns did not supply the resolution necessary for the separation. A base-deactivated Hypersil® column (250 × 4.6 mm, 5 μm) provided the necessary resolution and was selected for the chromatographic method.

It was necessary for the mobile phase for the HPLC method to separate L-768673 from process impurities, degradates as well as any microemulsion components which eluted with the drug through the solid-phase extraction column. An isocratic method was preferred over a gradient method. A mixed organic–aqueous mobile phase was adjusted to effect elution of the microemulsion components whilst retaining L-768673. An organic modifier, triethylamine (TEA), was added to improve the peak shape of the L-768673. This was complemented with the addition of trifluoroacetic acid (TFA) to maintain pH control for the separation. The final isocratic mobile phase consisted of acetonitrile–0.2% TFA, 0.1% TEA (53:47).

3.4. Chromatography

Good chromatographic profiles were obtained when the microemulsion samples were processed through the solid-phase extraction columns. This was verified by spiking placebo microemulsion with L-768673 and processing through the solid-phase columns. Fig. 2 shows sample chro-

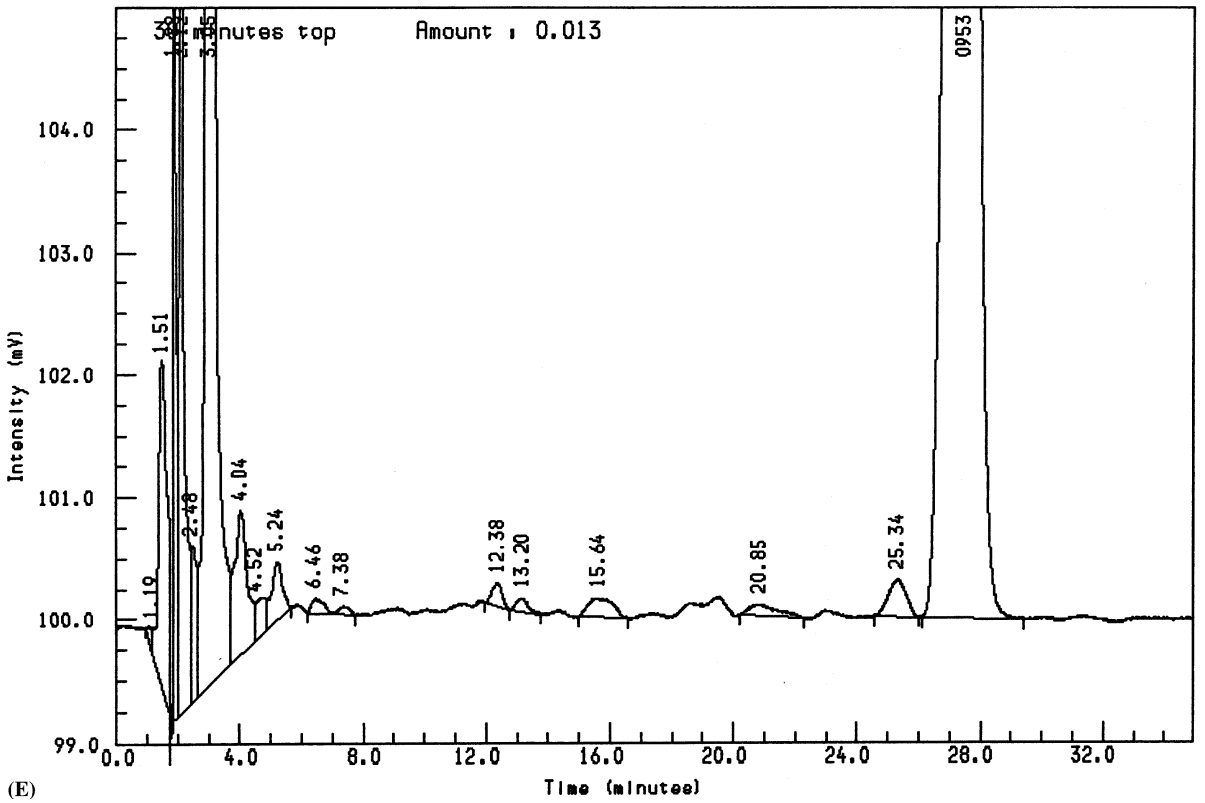


Fig. 2. (*Continued*)

matograms of: (A) L-768673 bulk material; (B) placebo microemulsion at the 0.1 mg ml^{-1} level; (C) placebo microemulsion at the 0.0125 mg ml^{-1} level; (D) 0.1 mg ml^{-1} microemulsion sample; (E) 0.0125 mg ml^{-1} microemulsion sample. The retention time for L-768673 was approximately 28 min at 1.5 ml min^{-1}.

3.5. Recovery and linearity

The recovery of L-768673 from the solid-phase extraction column as well as method precision were performed in the same experiment. Different amounts of a microemulsion formulation equivalent to 12.5, 50%, 75, 100, 125 and 150% of the 0.1 mg ml^{-1} assay concentration were extracted in duplicate. The data are shown in Table 1. The average recovery of the ten preparations over the concentration range was 99.1% with an RSD of 1.3%. This experiment not only demonstrated the consistent linearity of recovery of the drug in the microemulsion, but also demonstrated that the solid-phase extraction column is not overloaded in the normal operating range of the method.

The linearity ($R = 0.99976$) of the drug in solution was also demonstrated between 0.0001 and 0.202 mg ml^{-1}. This is 0.1–200% of the 0.1 mg ml^{-1} assay concentration (50 μl injection) and 0.5–1000% of the 0.01 mg ml^{-1} assay concentration (100 μl injection). Injection precision based on ten replicate injections of the 0.1 mg ml^{-1} standard was determined to be 0.09% RSD.

3.6. Selectivity

A sample solution of the placebo microemulsion was injected and exhibited no interference with the active peaks. Solutions of L-768673 standard in assay diluent were subjected to HCl/80°C, NaOH/80°C, H_2O_2/80°C, 80°C and 1000 lux light

Table 1
Recovery data from L-768 673 microemulsion

Level (%)	Theoretical (mg ml^{-1})	Assayed (mg ml^{-1})	% Recovery
150	0.1489	0.1478	99.3
150	0.1489	0.1449	97.3
125	0.1241	0.1228	98.9
125	0.1241	0.1207	97.2
100	0.09925	0.09779	98.5
100	0.09925	0.09818	98.9
75	0.07444	0.07417	99.6
75	0.07444	0.07434	99.9
50	0.04963	0.04888	98.5
50	0.04963	0.04942	99.6
12.5	0.01294	0.01291	99.7
12.5	0.01263	0.01292	102.3
Average (%)			99.1
% RSD			1.3

stressing, and assayed. No degradation was observed from light stressing. The 80°C stressed aliquot exhibited very small peaks ($<0.1\%$) at 0.57RRT and 0.76RRT. The H_2O_2/80°C aliquot exhibited a 6% area peak relative to the 100% standard at 0.64RRT, 0.3% at 0.37RRT and 0.3% at 0.42RRT. The HCl/80°C aliquot showed a 6% peak at 0.57RRT. The NaOH/80°C aliquot degraded rapidly with an 85% peak at 0.57RRT and an 8% peak at 0.75RRT. Data indicates the primary hydrolysis degradate at approximately 0.57RRT with a secondary degradate at 0.75RRT. The major oxidation degradate elutes at 0.64RRT. The method separates all observed degradates from the main L-768673 peak. The method also separates all process impurities from the main peak.

3.7. Limit of quantitation and detection

The limit of quantitation for the method has been determined as 0.1 μg ml^{-1} based on a signal to noise (S:N) ratio of greater than or equal to 10. This is 0.1% of the 0.1 mg ml^{-1} assay concentration and 0.5% of the 0.01 mg ml^{-1} assay concentration. Low level injection precision was performed at the limit of quantitation. Ten replicate injections of a 0.1% (0.1 μg ml^{-1}) standard of L-768673 had an RSD of 3.5% and ten injections of a 0.2% standard had an RSD of 2.4%. The limit of detection was determined as 0.05 μg ml^{-1} based on a S:N ratio of greater than or equal to 3.

3.8. Application

This method was applied to several stability formulations. Initial assay results were satisfactory but slightly low (97% of claim). Testing after one month at accelerated stability stations gave similar results. It was unclear whether the lower than expected results were real, or due to instability during sample preparation, low recoveries be-

Table 2
Comparison of solid-phase extraction (SPE) and DMA assays

Sample	%Claim (SPE)	%Claim (DMA)	% Difference (DMA-SPE)
Initial A	97.0	98.0	1.0
Initial A	96.5	100.5	4.0
Initial B	100.3	100.6	0.3
Initial B	100.3	100.7	0.4
1 MO A-25°C	97.0	96.4	−0.6
1 MO A-25°C	97.7	95.7	−2.0
1 MO A-30°C	97.8	96.9	−0.9
1 MO A-30°C	97.9	96.6	−1.3
1 MO A-40°C	97.3	96.5	−0.8
1 MO A-40°C	97.5	97.2	−0.3
Average %difference			0.0
SD-%difference			1.7

cause of matrix adsoption or lack of chromatography ruggedness.

It was decided to compare the equivalency of the solid-phase extraction method with the gradient method using DMA as the diluent. Five samples, two initials and three stability samples were assayed in duplicate. Table 2 shows that the average difference between the methods was 0.0% and the SD of the differences was 1.7%. The methods were considered equivalent, thus proving the initial assay values to be correct.

4. Conclusions

A sensitive, accurate and reliable stability indicating HPLC method employing solid-phase extraction for sample preparation was developed and validated for the quantitation of L-768673 in a microemulsion formulation. This method is able to selectively extract and separate L-768673 from the microemulsion components with excellent recoveries. This method was successfully applied to both release and test stability formulations.

The solid-phase extraction method has a significantly lower limit of quantitation than the equivalent gradient HPLC method in which DMA is employed as the diluent. The solid-phase extraction method also removes many of the emulsion matrix components, providing better selectivity for process impurities and degradates.

References

[1] K. Yamazoe, T. Horiuchi, T. Sugiyama, Y. Katagiri, J. Chromatogr. A 726 (1996) 241–245.
[2] L.C. Bailey, K.T. Tang, B.A. Rogozinski, J. Pharm. Biomed. Anal. 9 (1991) 501–506.
[3] A.W. Smallwood, K.E. DeBord, L.K. Lawyer, J. Anal. Toxicol. 16 (1992) 10–13.
[4] H. Chew, W. Jun, J. Pharm. Biomed. Anal. 15 (1996) 241–250.
[5] T. Okamura, S. Hirano, S. Wataru, T. Sakai, Chem. Express 7 (1992) 373–376.