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Determination of homotaurine as impurity in calcium acamprosate by capillary zone electrophoresis

H. Fabre[a,*], C. Perrin[a], N. Bosc[b]

[a]*Laboratoire de Chimie Analytique, Faculté de Pharmacie, F-34060 Montpellier Cédex 2, France*

[b]*Lipha S.A., Avenue Lacassagne, F-60003 Lyon, France*

Abstract

A method is reported which allows the quantification of homotaurine as an impurity in the drug. After addition of taurine as an internal standard, the sample is derivatised with fluorescamine at ambient temperature in 10 m*M* borate buffer, pH 9.2. The analytes are separated by capillary zone electrophoresis in a 31.2 cm (21 cm to the detector)×100 μm I.D. fused-silica capillary at a potential of +7 kV and 25°C. A 40 m*M* borate buffer, pH 9.2, is used as the electrolyte and detection is carried out at 205 nm. The validation tests showed that the method is reliable between 0.01% and 0.15% (m/m) of homotaurine with respect to the active drug. The limits of quantitation (0.01%, m/m) and detection (0.004%, m/m) allows to control the homotaurine content of the drug substance for which the maximum tolerated level is 0.05% (m/m). The proposed procedure (derivatisation and separation) developed in CE is rapid (20–25 min) by comparison to that currently used in HPLC (75 min). Satisfactory agreement was found between several batches of acamprosate analysed by CE and HPLC.

Keywords: Pharmaceutical analysis; Homotaurine; Taurine; Calcium acamprosate; Acamprosate

1. Introduction

Calcium acamprosate or calcium acetylhomotaurinate, $[CH_3–CO–NH–(CH_2)_3–SO_3]_2–Ca$ is a drug which is administered in tablet form to maintain abstinence in alcohol-dependent patients. A monograph on calcium acamprosate is being introduced by Lipha in the European Pharmacopoeia. The methods currently used by Lipha involve a potentiometric titration for the assay of the drug and an HPLC method for the determination of homotaurine $NH_2–(CH_2)_3–SO_3H$ which is both a precursor of the synthesis and a potential degradation product of acamprosate. In this method, homotaurine is derivatised off-line with fluorescamine. Separation is carried out on a C_{18} stationary phase under isocratic conditions with a run time of 45 min due to late-eluting by-products formed during the reaction. We previously showed that capillary electrophoresis could be used for the determination of calcium as a counter-ion, and for the determination of inorganic impurities in the drug substance [1,2]. This paper discusses the quantitative aspects of a capillary electrophoresis method for the determination of homotaurine to confirm the quality of the drug. A method was developed with the objective of quantifying homotaurine at a concentration level lower than 0.05% (m/m) with respect to the active drug, which is the maximum tolerated level of homotaurine in calcium acamprosate.

*Corresponding author.

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2. Experimental

2.1. Chemicals

Calcium acamprosate and homotaurine (HT) were kindly provided by Lipha (Lyon, France). Taurine (T) 99% purity and fluorescamine were from Sigma (Buchs, Switzerland), sodium tetraborate·$10H_2O$ was from Merck (Darmstadt, Germany). Other chemicals were of analytical grade. Water, doubly distilled in a glass apparatus, was used throughout.

2.2. Solutions

2.2.1. Electrolyte solution

The background electrolyte solution was 40 m*M* tetraborate sodium solution in water with a natural pH of 9.2. The solution was stored at 4°C and used within one month.

2.2.2. Fluorescamine solution

Fluorescamine solution was prepared by dissolving 3 mg of fluorescamine in 1 ml of acetone containing 20 μl of pyridine. The solution stored at 4°C was used within one day.

2.2.3. Stock solutions of sodium tetraborate in water

Stock solutions of sodium tetraborate (10 m*M*) in water, calcium acamprosate (20 g/l), homotaurine (200 mg/l) and taurine (200 mg/l) in 10 m*M* sodium tetraborate were prepared. The solutions stored at 4°C were used within fifteen days.

2.2.4. Working standard and test solutions

Working standard and test solutions were daily prepared in 4 ml capped vials. A standard solution of homotaurine was prepared by adding a 50 μl aliquot of homotaurine and taurine stock solutions to 1.15 ml stock tetraborate solution. A test solution of acamprosate was prepared by adding a 50 μl aliquot of taurine stock solution and 1 ml aliquot of acamprosate stock solution to 200 μl tetraborate stock solution.

2.2.5. Derivation of the working solutions with fluorescamine

An aliquot of 25 μl of fluorescamine solution was added to 150 μl of standard and test solutions, respectively. The resulting solutions were vortexed for 1 min then transferred in conical vials of 200 μl for analysis and placed in the sample tray cooled to 10°C in the electrophoresis apparatus.

Table 1
Operating conditions

Rinse	2 min, methanol
Wait	0.10 min, water
Rinse	3 min, 1 *M* sodium hydroxide
Wait	0.10 min, water
Rinse	2 min, electrolyte
Wait	0.10 min, water
Sample injection	5 s, 0.2 p.s.i., anodic side
Wait	0.10 min, water
Separation	15 min; +7 kV; 25°C
Detection	205 nm; bandwith 20 nm; acquisition rate 4 Hz; filter normal; <16 points

2.3. Apparatus and operating conditions

CE experiments were performed on a Beckman (Palo Alto, CA, USA) P/ACE MDQ instrument equipped with a diode array detector. Separations were carried out in a fused-silica capillary (TSP, Composite Metal Services, Hallow, UK), 31.2 cm (21 cm to the detector)×100 μm internal diameter, housed in a cartridge with a detection window 100×800 μm. Prior to its first use, the capillary was pre-conditioned for 20 min with 0.1 *M* sodium hydroxide, and then for 3 min with water. Conical vials of 200 μl were used for the samples; other vials were of about 2 ml volumes. The operating conditions used for each run are given in Table 1.

Standard and test solutions were placed in a bracketed sequence and each solution was injected in duplicate. Relative corrected peak areas (area/migration time) of homotaurine/taurine and relative migration times homotaurine/taurine were used. The separation vials were changed after two injections.

3. Results and discussion

3.1. Preliminary investigations

Homotaurine possesses two ionisable functional

groups, but no chromophore allowing direct UV detection. Indirect detection was initially investigated as it is more readily executed and does not require a derivatisation step.

3.1.1. Indirect UV detection

Based on the work of Bruin et al. [3] on indirect UV detection of amino acids, sodium salicylate was used as visualisation agent in the electrolyte. The optimum conditions for separation and detection for a 2.5 g/l acamprosate solution were obtained using an electrolyte consisting of 4 m*M* sodium salicylate and 0.4 m*M* borate adjusted to pH 10.6 with sodium hydroxide; a potential of +15 kV; an injection time of the sample of 3 s under 0.3 p.s.i., and a detection wavelength of 205 nm. Under these conditions, HT and acamprosate were separated with respective migration times (t_m) of 1.30 min and 1.53 min. A satisfactory peak shape was obtained for homotaurine but its limit of quantification (LOQ) was only 0.8% (m/m) with respect to the drug substance, which was a lower level of discrimination than the required LOQ.

3.1.2. Detection after derivatization with fluorescamine

Derivatisation of the primary amino group was then investigated in order to lower the limit of detection. Among the derivatisation agents of amines commonly used for the derivatisation of amino acids in CE [4], peptides and proteins, fluorescamine [5,6] was selected as it is known to react very rapidly with primary amines, reaction with the latter yielding fluorescent compounds which absorb strongly in UV. Fluorescamine should be dissolved in an organic solvent as it is insoluble in water. However, hydrolytic solvents such as methanol cannot be used as fluorescamine is rapidly degraded. The nature of the solvent is very important for the kinetics of the reaction, as are the pH and nature of the buffer used for solubilising the sample. Acetone, acetonitrile and dioxane as solvents with sodium tetraborate or phosphate buffer, at pH ca. 9, to provide alkaline conditions for reaction, give the best results. The reaction is generally complete at ambient temperature after a few seconds and the stability of the derivatives appears to be of the order of several hours [5–7]. Despite the advantages of fluorescamine, to the best of our knowledge there are a few papers describing its use as derivatization agent for amino acids in CE [7,8].

3.1.3. Optimisation of the derivatization reaction

The derivatization protocol we developed is based on that used by Guzman et al. [7] for lysine and arginine, modified with respect to the volume fluorescamine added to the sample, in that it was reduced by 1/4 and the borate concentration of the medium was lowered from 100 to 10 m*M* to increase the stacking effect in the electrophoresis separation. Under these conditions, the response factor was found to be unchanged and the reaction was found to be immediate and complete at ambient temperature. An internal standard was also incorporated to the sample solution to compensate for small variations due to sample evaporation and possible fluctuations of the injection system. Among different amino acids, taurine was found to be the most suitable internal standard as its t_m is very close to that of HT. The derivatised analytes were found to be stable at least 1 h at ambient temperature and 12 h in the autosampler refrigerated at 10°C.

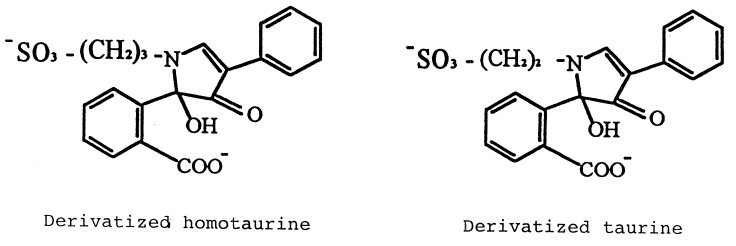

Fig. 1. Structure of derivatised homotaurine and taurine.

3.1.4. Optimisation of the separation conditions and limit of detection

3.1.4.1. Capillary diameter

The first experiments were carried out using a capillary of 75 μm internal diameter which gave a limit of detection (LOD) of 0.04% (m/m) whereas the use of a 100 μm I.D. capillary allowed this limit of detection to be lowered. Particular care was taken to avoid laminar flow and siphoning effects by adjusting the level of liquid in sample and separation vials.

3.1.4.2. Electrolyte concentration and separation potential

The selected electrolyte was sodium borate in water which has a natural pH of 9.2. At this pH, derivatised HT and T are doubly charged anions

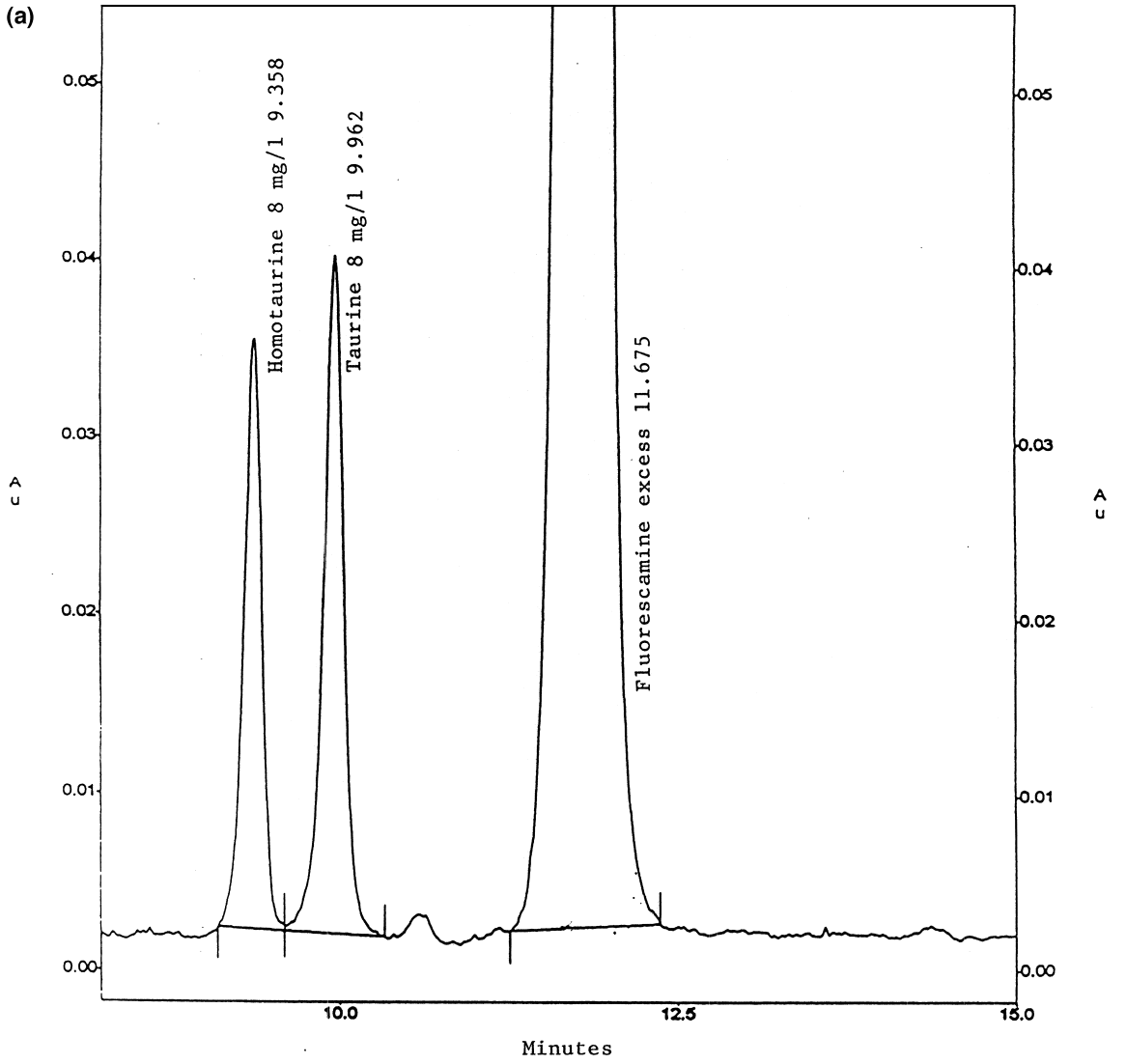

Fig. 2. Electropherograms obtained after derivatisation with fluorescamine in the presence of taurine (internal standard) of : (a) a standard solution of homotaurine [8 mg/l corresponding to 0.05% (m/m) of HT in the drug substance]; (b) a calcium acamprosate solution (16 g/l) without homotaurine; (c) an acamprosate solution (16 g/l) spiked with homotaurine at a 0.04% (m/m) level.

(Fig. 1). Since the test solution had a high ionic strength due to the high concentration of acamprosate, the borate concentration of the electrolyte had a significant impact on the resolution. An increase in the electrolyte concentration resulted in an increase in efficiency and a decrease in the LOD by a stacking effect, a point was reached when the Joule heating was not dissipated. The best compromise was obtained for a 40 m*M* borate concentration and a potential of +7 kV, which gave a current of 100 μA.

3.1.4.3. Injected volume and sample concentration

For the same injected amount, the resolution increased as the injected volume was decreased. The best result was obtained for an acamprosate concentration of 16 g/l and a 54 nl injected volume, corresponding to a pressure of 0.2 p.s.i. applied for 5 s. With the equipment used, these experimental conditions do not correspond to the optimum performance obtainable with regards to injection precision. However, the use of an internal standard

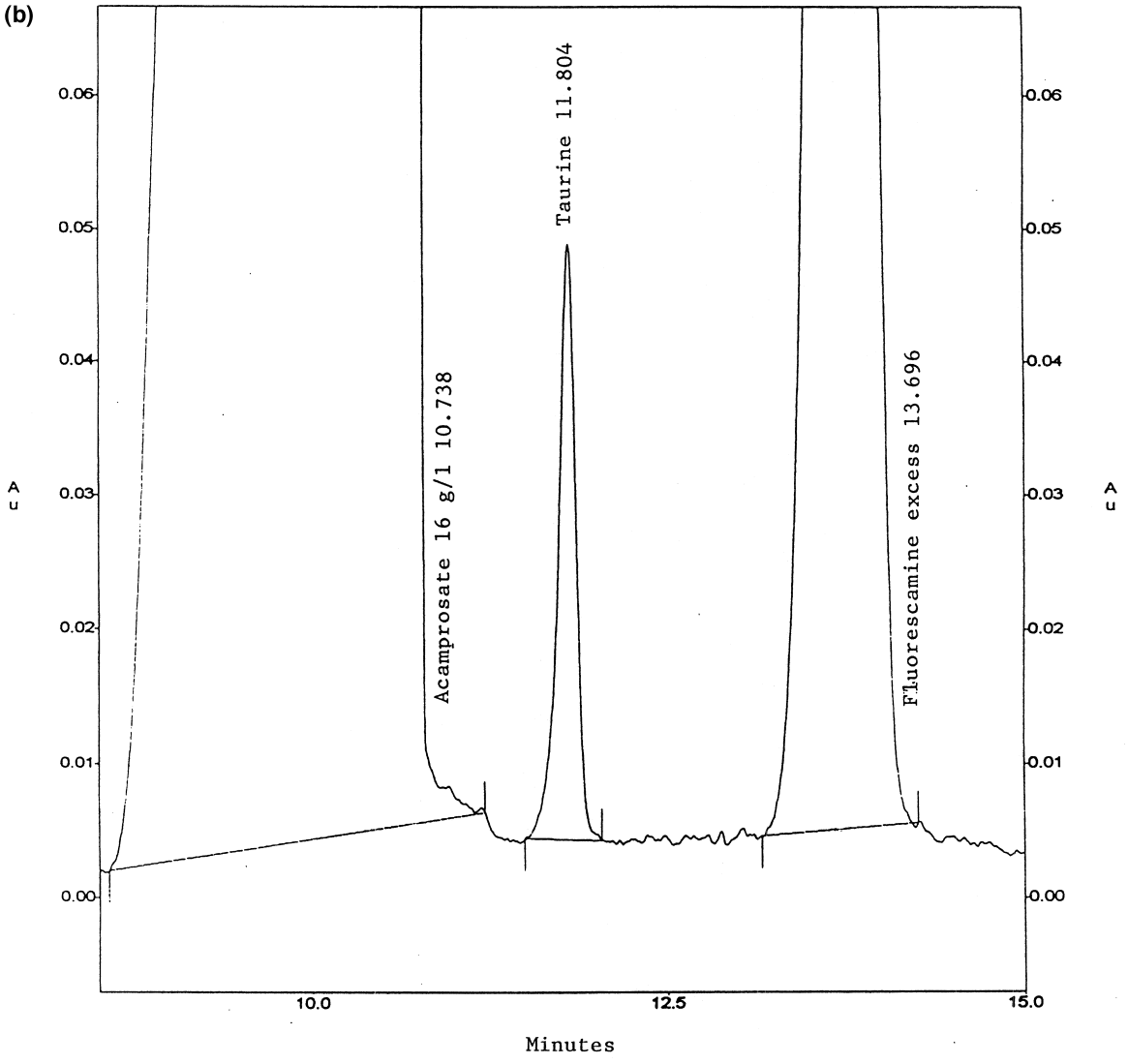

Fig. 2. (*continued*)

compensated for the small variations in the injection system.

3.1.4.4. Detection wavelength

The best signal-to-noise ratio was obtained at 205 nm with a spectral bandwith of 20 nm.

3.1.4.5. Capillary rinse between injections

Various rinse solutions were tested (hydrochloric acid, sodium hydroxide, methanol); the best performances were obtained using rinse cycles with methanol, sodium hydroxide and then buffer.

The final conditions selected are given above in Sections 2.2 and 2.3.

3.2. Method validation

The method was validated according the recommendations of the International Conference of Harmonisation [9].

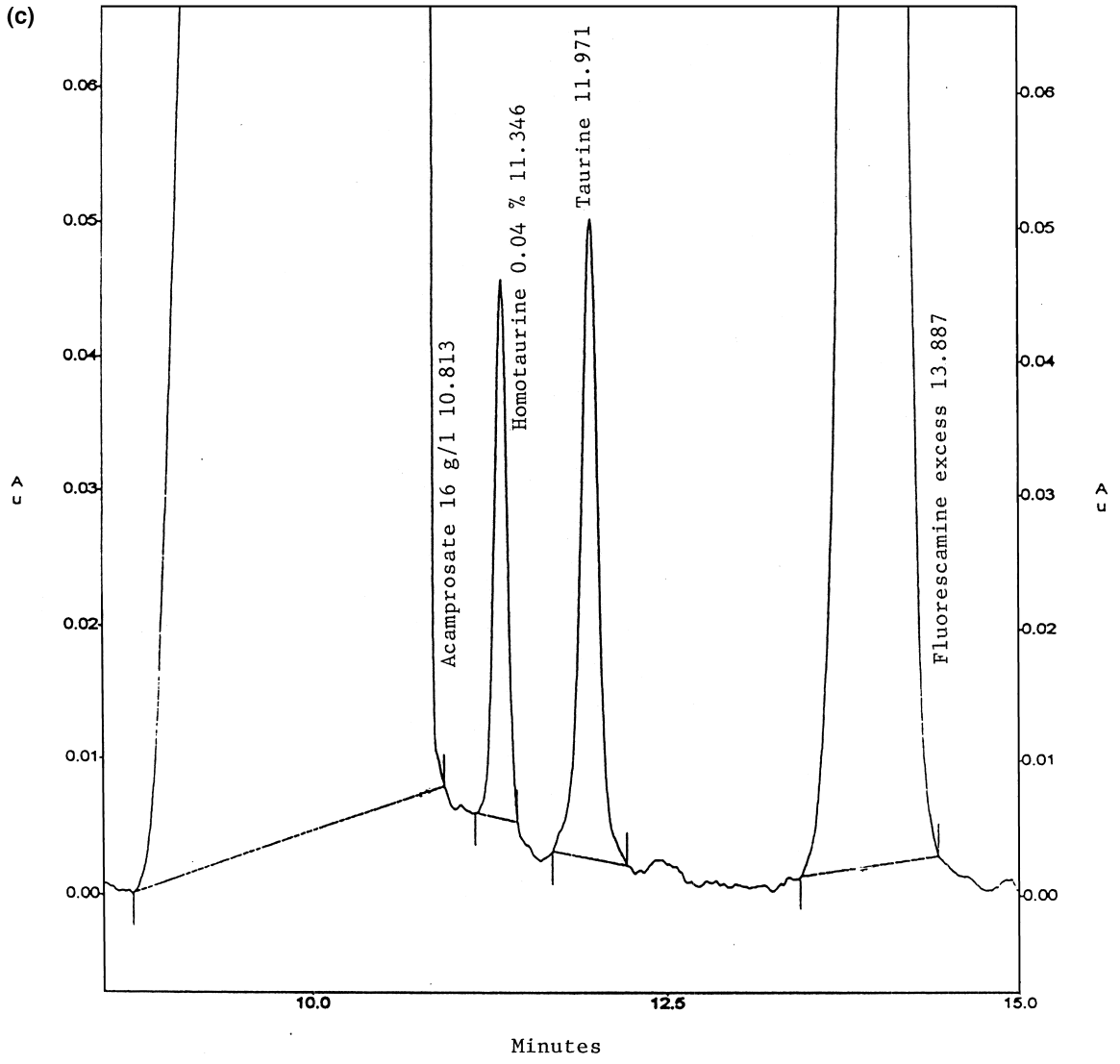

Fig. 2. (*continued*)

3.2.1. Specificity

Fig. 2 shows typical electropherograms of a standard solution of HT, of a calcium acamprosate solution without HT and an acamprosate solution spiked with HT at 0.04% (m/m) with respect to the drug substance. The increase in the t_m values of HT and T in the test samples can be explained by the high ionic strength of the sample which alters the conductivity in the capillary. However, HT can be readily be identified in a test sample by co-injection of HT or by calculating the relative migration time homotaurine/taurine (RMT), which approximately was 0.945 in all the samples. The ratio of corrected peak areas (CPA=peak area/t_m) of homotaurine/taurine must be used for the quantification of HT in test samples by reference to the standard solution. Internal standard and blank reagent solutions derivatised with fluorescamine did not give any interfering peak at the t_m values of the analytes.

3.2.2. Linearity of the calibration line

The linearity of the ratio of corrected peak areas homotaurine/taurine (RCPA) versus HT concentration was assessed with five standard solutions with variable HT concentrations covering the range 1.5–24 mg/l, which corresponded to 0.01–0.15% HT (m/m) with respect to calcium acamprosate. The concentration of T was kept constant (8 mg/l) in all the solutions.

The regression equation was:

RCA=−0.0093 (±0.015)+17.4568 (±0.1803) HT % with the confidence intervals calculated at $p=0.05$. The relationship was linear ($r^2=0$ 999) and went through the origin which allowed the routine use of one calibration point.

3.2.3. Linearity of the procedure and recovery studies

The linearity of the procedure was assessed by performing three series of determinations over three days, each time at five concentration levels of HT. All solutions and reagents were independently prepared on each occasion. On each of the three days, a solution of acamprosate (without HT) spiked at five concentration levels of HT in the range 0.01–0.15% (m/m) and a calibration solution of HT corresponding to at 0.05% (m/m) were prepared. Each solution was injected in duplicate and recovery studies were calculated using RCPA. The results presented in Table 2, demonstrate that acceptable recovery data were obtained over the concentration range with a mean relative error lower than 1.62%. An ANOVA (analysis of variance) carried out on % recoveries showed that variances were homogeneous and there was no 'day' effect. The data obtained in each series were grouped to plot a graph of amount found of HT (3*y*) versus amount added of HT (1*x*). For this purpose, the HT concentrations were adjusted to a unique mean concentration *x* at each level and the value of *y* was accordingly corrected. The regression equation was :

HT found=0.0002 (± 0.0002) +1.028 (± 0.0275) HT added with $r^2=1$. The relationship was found to be linear; the slope did not differ significantly from unity and went through the origin ($p=0.05$) which showed that the procedure was accurate.

Table 2
Linearity and accuracy of the procedure: recovery studies for homotaurine in calcium acamprosate

Day	Homotaurine added (%, m/m)	Homotaurine found (%, m/m)	Recovery (%)
1	0.0100	0.0101	101.38
	0.0200	0.0204	102.01
	0.0500	0.0506	101.26
	0.1000	0.1015	101.47
	0.1500	0.1517	101.15
2	0.0102	0.0103	101.46
	0.0203	0.0207	102.18
	0.0500	0.0514	101.72
	0.1000	0.1013	101.25
	0.1500	0.1518	101.16
3	0.0103	0.0104	101.20
	0.0206	0.0209	102.53
	0.0500	0.0511	102.12
	0.1000	0.1020	102.00
	0.1500	0.1521	101.40

3.2.4. Precision

The repeatability of the system (injection, separation and peak area integration) was assessed by injecting standard and test solutions of acamprosate spiked with 0.01, 0.02 and 0.15% of HT on six successive occasions. For example, relative standard deviations (RSDs) for test solutions of HT in acam-

Table 3
Repeatability of test solution preparation (n=6)

	Acamprosate spiked with					
	0.01% (m/m) HT		0.02% (m/m) HT		0.15% (m/m) HT	
	Mean	RSD %	Mean	RSD %	Mean	RSD %
RMT	0.947	0.30	0.946	0.34	0.949	0.26
RCPA	0.165	11.65	0.323	8.56	2.568	4.45

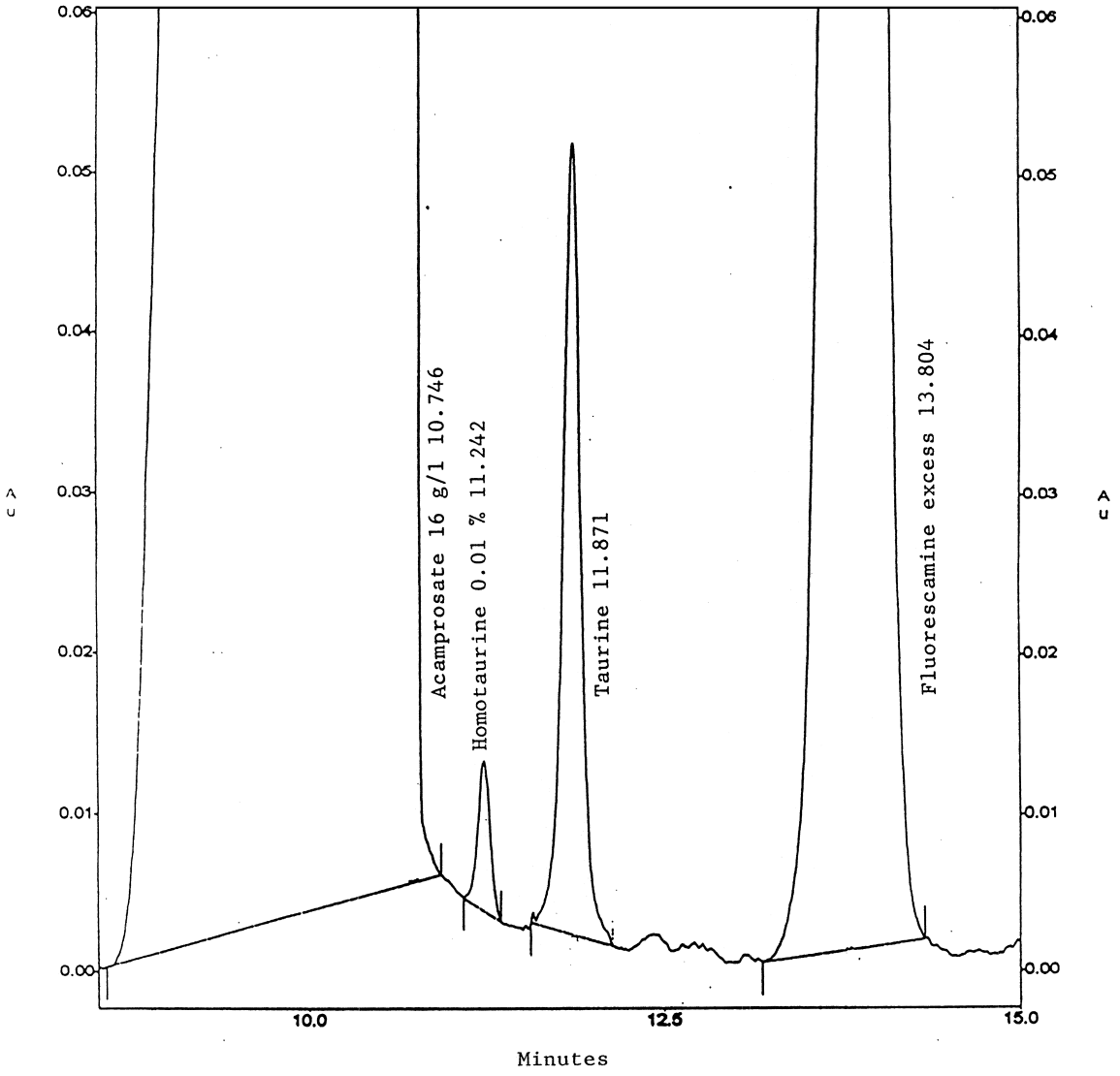

Fig. 3. Electropherogram from an homotaurine solution at the LOQ (0.01% m/m).

prosate at 0.01% were 0.65% for RMTs and 3.98% for RCA. Similar results were obtained for other solutions.

Repeatability of test and calibration preparation is important when a derivatisation step is involved in a procedure. The repeatability of preparation of a standard solution (0.05%) and test solutions (0.01, 0.02 and 0.15%) was assessed by preparing independent solutions of each, on six occasions. The results obtained for test solutions are given in Table 3.

Inter-day intermediate precision at different concentrations of HT was evaluated from the results of recovery studies. Table 2 shows that inter-day variations of the overall procedure are acceptable at the different concentrations tested.

3.2.5. Limit of quantification (LOQ)

At the LOQ, a RSD around 10% and an accuracy of ±10% is generally tolerated for impurities. A sample of calcium acamprosate spiked with 0.01% (m/m) of HT gave a recovery of 101.17% with a

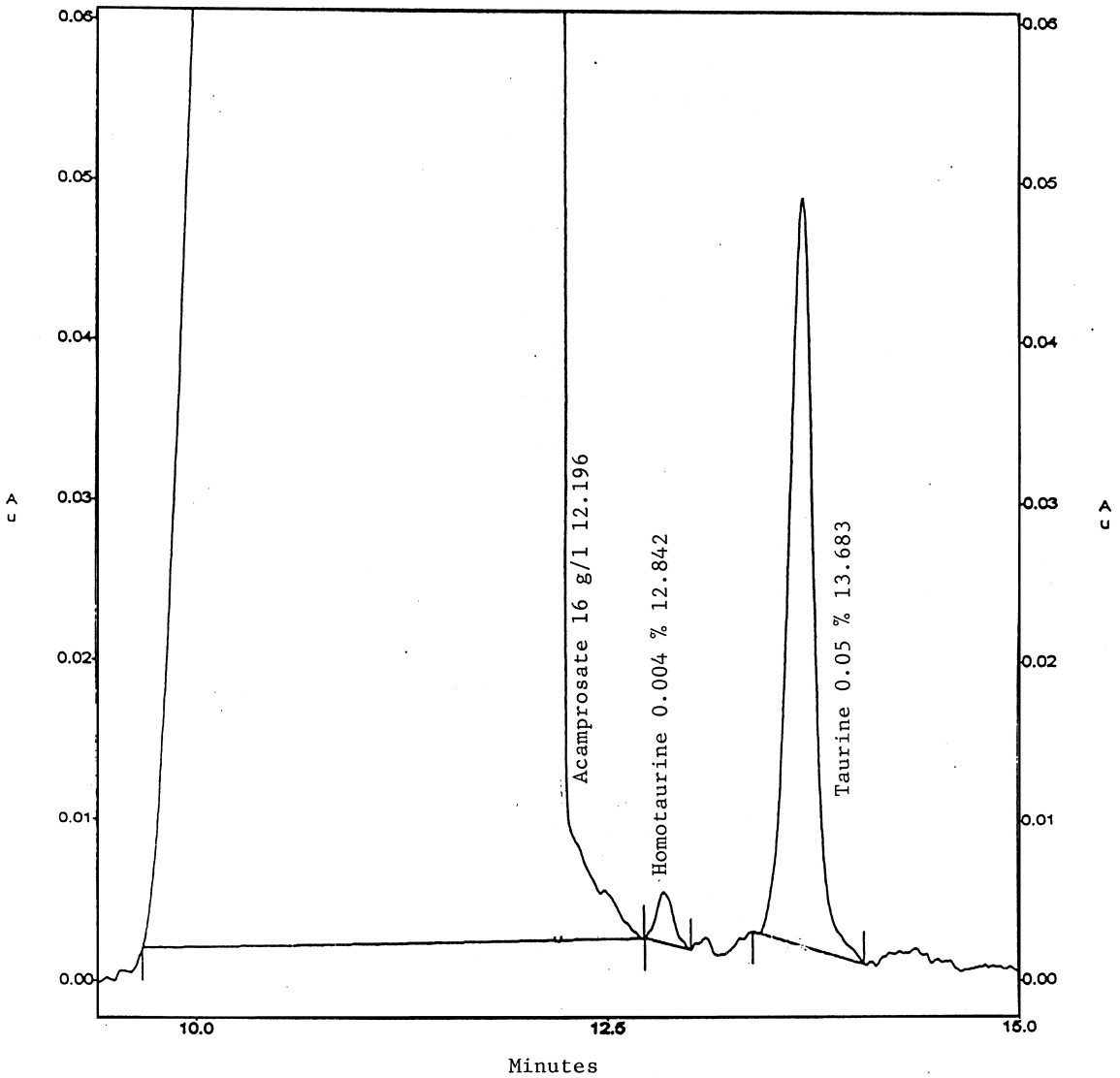

Fig. 4. Electropherogram from an homotaurine solution at the LOD (0.004%, m/m).

RSD of 11.65% ($n=6$ preparations) and was considered as the LOQ experimentally determined. Fig. 3 shows the electropherogram at the LOQ.

3.2.6. Limit of detection (LOD)

The LOD experimentally determined (signal/noise=3) was about 0.004% (m/m). The corresponding electropherogram is given in Fig. 4.

3.2.7. Application to real samples

Five batches of calcium acamprosate were analysed for HT content and the results were compared to those found using the HPLC currently used by Lipha. Homotaurine was not detected either by CE or by HPLC (LOD 0.001% determined by HPLC on a standard solution) in three batches. Homotaurine was found to be present at a 0.04% m/m (0.04; 0.04) by CE and 0.04% by HPLC in batch X, and 0.010% (0.010; 0.011) by CE and 0.01% in HPLC in batch Y. The agreement of the results between these two orthogonal methods allow the confirmation of the HT content.

4. Conclusion

A capillary electrophoresis method has been developed for the determination of homotaurine in calcium acamprosate. The method has been validated and the results showed it gives acceptable performances between 0.01 and 0.15% (m/m) of HT with respect to the drug substance. The method gives a LOQ of 0.01% (m/m) of HT which allows the determination of whether the drug substance complies with the specifications stated (maximum tolerance 0.05%, m/m of HT). The protocol (derivatisation and separation) developed is rapid (20–25 min) by comparison to that currently used in HPLC which requires 75 min. The agreement between the two methods confirms that CE is a useful complementary or alternative technique to HPLC for the analysis of impurities in pharmaceuticals.

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