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Determination of tramadol in various dosage forms by capillary isotachophoresis

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Abstract

Cationic capillary isotachophoresis (ITP) with conductometric detection has been used for separating and determining milligram amounts of tramadol [2-dimethylaminomethyl-1-(3-methoxyphenyl)-cyclohexanol hydrochloride] (I) in seven commercial mass-produced pharmaceutical preparations. The optimised ITP electrolyte system consisted of 5 mM potassium picolinate + 5 mM picolinic acid (pH 5.25) as the leading electrolyte and 10 mM formic acid as the terminating electrolyte. The driving and detection currents were 50 μA (for 320 s) and 10 μA, respectively (a single analysis took 12–15 min). Under such conditions the effective mobility of I was determined as 24.26×10^{-9} $m^2\ V^{-1}\ s^{-1}$ (with tetraethylammonium ion as standard); thermodynamic pK_a value of I was 9.44 ± 0.03 ($n = 8$) as determined by UV spectrophotometry at 25°C and $I = 0.01$ (NaCl). The calibration graph relating the ITP zone length to the concentration of I was rectilinear ($r = 0.99997$) in the range 15–180 mg l^{-1} of I. The relative standard deviation (RSD) was 0.21% ($n = 6$) when determining 60 mg l^{-1} of I in pure test solution. Sample pre-treatment of the dosage forms involved dilution or extraction of I with water (for suppositories the extraction was carried out in an ultrasonic bath at 40°C for 10 min). The method was suitable for determining 50 or 100 mg ml^{-1} of I in injections and drops, 50 mg of I in capsules, and 100 mg of I in suppositories with RSD values 0.4 to 1% ($n = 6$). According to the validation procedure based on the standard addition technique the recoveries were 97.2–100.1% of I.

Keywords: Tramadol; Isotachophoretic separation; Pharmaceutical formulations</kwdg*

1. Introduction

Tramadol [(±)trans-2-(dimethylaminomethyl-1-(3-methoxy-phenyl)-cyclohexanol hydrochloride] (I) (Fig. 1) is a centrally acting analgesic-anodyne agent of high oral bioavailability. As a relatively new drug it is not yet included in internationally recognised pharmacopoeias though various mass-produced dosage forms containing I are available on the market. The I contains a weakly absorbing chromophore in its molecule and it was determined by HPLC with UV detection [1–3] or fluorescence detection [4] in pharma-

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ceuticals [1] or urine [2] or blood plasma [3,4]. Gas chromatography with a nitrogen-selective detector [5] or gas chromatography-mass spectrometry [6] were also used for determining I in human blood. To our best knowledge, no attempts have yet been made to determine I by any electro-migration separation method.

Generally basic amines (B) are completely ionised to form stable cationic species (BH^+) in acid aqueous solutions if the condition: $(pH)_{solution} \leq (pK_a)_{base} - 2$ is fulfilled. For I as a tertiary amine comprising two methyl groups and one relatively bulky substituent, one should expect $pK_a \approx 9.5$ considering general rules of the chemical structure-basicity relations. The basic character of I should facilitate the formation of cationic species in a slightly acidic medium and hence to make the determination of I by capillary isotachophoresis (ITP) or capillary zone electrophoresis feasible. Since we could not find any credible pK_a data on I in the literature, we decided to determine initially its pK_a value by a reliable experimental method (UV spectrophotometry) as the preliminary step. The major task was to find optimum system of operational electrolytes to ensure correct ITP migration of I, to calculate its effective mobility and to examine the calibration dependencies necessary for the ITP determination of milligram amounts of I in pharmaceutical formulations including solid dosage forms, injections, drops and suppositories.

2. Experimental

2.1. Materials

The standard of tramadol hydrochloride lot SK-I-8902 was obtained from PRO.MED.CS (Prague, Czech Republic). Other chemicals (formic acid, hydrochloric acid, sodium hydroxide, potassium hydrogencarbonate and tetraethylammonium iodide purchased from Lachema Brno; picolinic acid purchased from Fluka AG Buchs) were of analytical grade. A Millipore Milli-Q RG ultra-pure water was used throughout. The dosage forms analysed were Tramal capsules, Tramal drops, Tramal suppositories, Tramal 50 injections and Tramal 100 injections (all obtained from Léčiva Praha), MABRON capsules (Medochemie Ltd, Limassol, Cyprus) and PROTRADON capsules (PRO.MED.CS, Prague)-for the nominal content of the labelled I see Table 2.

2.2. Instrumentation

Isotachophoretic analyses were carried out with use of a computer-controlled EA 100 ITP analyser (VILLA s.r.o., Spišská Nová Ves, Slovak Republic) operated in the single-column mode. The analyser was equipped with a 30-μl sampling valve, a 120×0.3 mm (i.d.) analytical capillary made of fluorinated ethylene–propylene copolymer and a conductivity detector. Quantitative data were obtained by off-line processing of the stored isotachophoregrams providing the length of the isotachophoretic zones by using the appropriate ITP software package supplied by the VILLA company.

For the spectrophotometric determination of the ionisation constant pK_a of I the Hewlett-Packard 8453 UV/VIS spectrophotometer equipped with a DAD and a thermostated cell holder housing a 3.5-cm fused silica cell (volume 100 ml) was employed. A Radiometer PHM-64 pH meter with a GK-2041-B combined glass electrode calibrated with standard buffers (hydrogen phthalate pH 4.01, phosphate 6.88 and borate 9.22) was used for the pH measurements.

Bandelin SONOREX RK 100 ultrasonic bath (Berlin, FRG) was employed for the sonication of solid dosage forms to facilitate the dissolution or extraction of I.

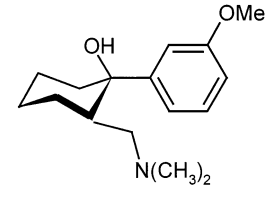

Fig. 1. Chemical structure of tramadol base.

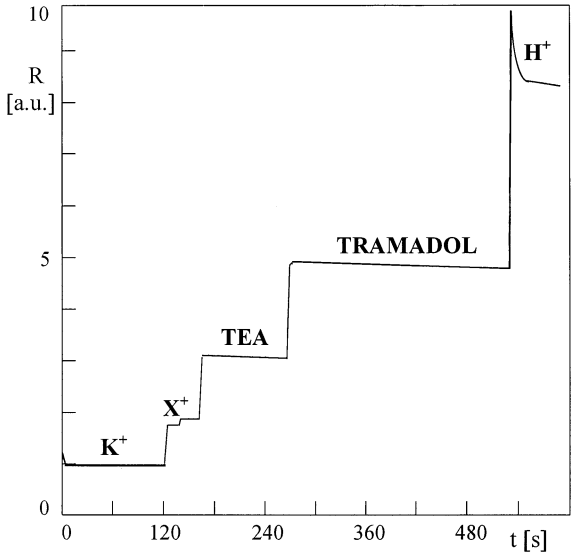

Fig. 2. Isotachophoregram of 0.4 mM I and 0.2 mM tetraetylammonium (TEA) as the mobility standard; operational system: 5 mM picolinic acid + 5 mM K picolinate (LE) and 10 mM formic acid (TE); X^+, unidentified impurities; time counting started after switching the driving current 50 μA (320 s) to 10 μA; *R*, resistance (in arbitrary units).

2.3. Determination of pK_a of I

A solution of I (volume 100 ml, c(I) = 0.03–0.06 mM; ionic strength $I = 0.01$ adjusted with NaCl) placed in the 3.5-ml fused silica cell thermostated at 25 ± 0.2°C was titrated with μl volumes of 0.1–1M NaOH directly in the spectrophotometric cell and the pH and absorption curves were measured simultaneously as described earlier [7]. The data of the absorbance (A) versus pH curves measured at 210 and 220 nm (corrected for the absorption of OH^-) and involving typically 15 to 25 experimental points were used to compute [8] the thermodynamic pK_a constant [9] of I by non-linear regression. All the A-pH curves were measured in quadruplicate and the final pK_a was calculated as the arithmetic mean of the eight individual results.

2.4. The effective mobility and calibration curve of I

The measurement of the effective mobility of I was carried out with 0.4 mM solution of I and 0.2 mM tetraethylammonium (TEA) iodide as the standard of mobility. The effective mobility of the drug was calculated from the relative waveheights [10] (see also the isotachophoregram in Fig. 2) taking into account the tabulated values of ionic mobilities of K^+ and TEA of 76.1×10^{-9} and 33.8×10^{-9} m^2 V^{-1} s^{-1}, respectively.

The calibration curve was measured with 0.05 to 0.6 mM I solutions (five concentrations, each measured in triplicate) corresponding to 15–180 mg l^{-1} of I. The time (t, in seconds) of the passage of the zone of I through the detector was read as the quantitative parameter (obtained by off-line computer-aided processing of the stored isotachophoregrams) and the $t = f$ [c(I)] curve was

Table 2
ITP determination of tramadol in pharmaceutical preparations

Formulation	I Nominal content	ITP method Content ± RSD[a] mg piece^{-1} or mg ml^{-1}
Tramal capsules	50 mg capsule^{-1}	46.26 ± 0.96
Mabron capsules	50 mg capsule^{-1}	47.73 ± 0.82
Protradon capsules	50 mg capsule^{-1}	48.05 ± 0.94
Tramal drops	100 mg ml^{-1}	96.88 ± 0.91
Tramal 50 injections	50 mg ml^{-1}	51.47 ± 0.62
Tramal 100 injections	50 mg ml^{-1}	51.18 ± 0.68
Tramal suppositories	100 mg suppository^{-1}	101.42 ± 0.52

[a] Six replicate results.

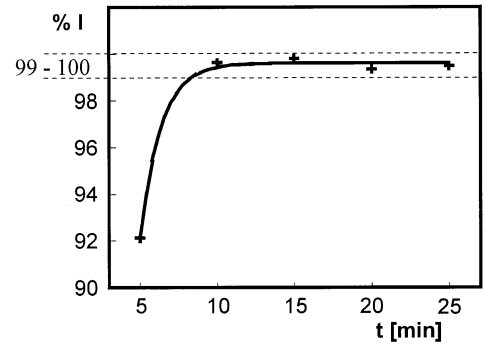

Fig. 3. Time dependence of the yield of extraction of I into water from Tramal suppository sonicated at 40°C.

evaluated by linear regression. The driving and detection currents were 50 μA (for 350 s) and 10 μA, respectively. The leading electrolyte (LE) was a buffer solution containing 5 mM picolinic acid and 5 mM potassium picolinate (pH = 5.25). The terminating electrolyte (TE) of pH 2.58 was 10 mM in formic acid (terminating ion H^+). The electrolytes were degassed ultrasonically before use.

2.5. Analysis of pharmaceutical preparations

All dosage forms examined were processed in such a way that the concentration of I in the final test solution or extract fell within the cited ITP calibration range. Since the I is readily soluble in water, the injections and drops were just diluted with water to achieve the appropriate concentration.

2.5.1. Capsules

The contents of ten capsules were emptied into an agate mortar, homogenised and an appropriate amount of the powdered material corresponding to ≈100 mg of I was weighed and dissolved in ≈200 ml of water by applying a 10-min sonication; thereafter the solution was diluted to 250 ml with water, filtered through a dry paper, 30 ml of the filtrate was diluted to 100 ml with water and analysed by ITP.

2.5.2. Suppositories

Five suppositories were finely grated on a stainless steel grater and the splinters were homogenised. An amount of the homogenised mass corresponding to 100 mg of I was weighed, treated with 250 ml of warm water (40°C) in a conical 500-ml flask and the suspension was sonicated for 10 min at 40°C; a 5.0-ml aliquot of the sonicated solution was diluted to 50 ml with water and the I was assayed by ITP.

2.5.3. Liberation of I from melted suppositories

An amount of the homogenised suppositories corresponding to 100 mg of I was weighed and treated with 250 ml of warm water (40°C) in a conical 500-ml flask; the suspension was sonicated for 25 min at 40°C; 5-ml aliquots of the sonicated solution were taken from the flask in 5-min intervals, each aliquot was diluted to 50 ml with water and these solutions were analysed by ITP to find minimum time needed for complete liberation of I from the melted suppository.

2.6. Accuracy test

All the pharmaceutical formulations were initially analysed by ITP as described above; thereafter the samples were treated with known amounts of tramadol standard (to double the concentration of I in the final test solution) and again subjected to ITP assay. The recoveries of the added amount of I were calculated.

3. Results and discussion

3.1. The pK_a value of tramadol

The thermodynamic pK_a value of I equals to 9.44 ± 0.03 ($n = 8$) as determined by UV spectrophotometry at 25°C and $I = 0.01$ (NaCl). This result indicates that the tramadol base will be practically completely ionised to BH^+ species at pH ≤ 7.5.

3.2. Selection of operational electrolyte system

The ITP operational system has been optimised with respect to the quality of separation, the sensitivity of the ITP determination and time of analysis. Various slightly acid operational systems with K^+ as the leading ion and acetate and/or picolinate as the counter ion have been tested. The optimisation involved critical selection of the kind and concentration of the counter-ion and, consequently, the pH of the leading electrolyte (LE). The parameters of the operational electrolyte systems are shown in Table 1. It can be clearly seen that the picolinate system of pH 5.25 shows best ITP parameters as for the values of the driving and detection currents (50 and 10 μA, respectively), duration of analysis (≈7.5 min after switching from the driving to the detection current) and sensitivity (the length of the zone of 0.4 mM I is 259 s—compared to 50 s for the corresponding acetate system). The effective mo-

Table 1
Effect of concentration of leading electrolyte on the migration of 0.4 mM I and fundamental ITP characteristics; TE: 10 mM formic acid[a]

$c(K^+)$ (mol l^{-1})	$c(R^-)$ (mol l^{-1})	I (μA)	t (s)	pH_{LE}	(m^2 V^{-1} s^{-1})	Shape/length of I wave (s)	Duration of analysis (s)[b]
Picolinate as the counter ion R							
		50	320			Regular, slightly descending/259	
0.005	0.01	10	600	5.25	24.26 ± 0.10		442
		50	400			Regular, slightly descending/269	
0.01	0.02	10	600	5.20	22.47 ± 0.09		642
Acetate as the counter ion R							
		75	300			Regular, rectangular/50	
0.005	0.01	20	900	4.56	23.33 ± 0.12		460
		75	600			Regular, rectangular/180	
0.01	0.02	20	900	4.50	22.15 ± 0.07		812

[a] $c(K^+)$, concentration of the leading ion; $c(R^-)$, concentration of the counter-ion; I, driving or detection current; t, time of ITP run; pH_{LE}, experimental pH of the leading electrolyte; , effective mobility of I; s, standard deviation ($n = 6$).

[b] After switching from driving to detection current.

Table 3
ITP recoveries of I added to pharmaceutical formulations

Formulation	I added (mg l^{-1})	I found ± RSD[a] (mg l^{-1})	Recovery (%)
Tramal capsules	60	59.21 ± 0.93	98.68
Mabron capsules	60	58.34 ± 0.74	97.23
Protradon capsules	60	58.66 ± 0.82	97.77
Tramal drops	60	59.48 ± 1.17	99.13
Tramal 50 injections	60	60.01 ± 0.45	100.02
Tramal 100 injections	60	60.03 ± 0.42	100.05
Tramal suppositories	40	39.98 ± 0.53	99.95

[a] Six replicate results

bility of I that amounts to 24.26×10^{-9} m^2 V^{-1} s^{-1} (cf. Table 1) is in fact the ionic mobility value of I since the I is completely ionised in the electrolyte system used. A typical isotachophoregram of I recorded with the optimum LE is shown in Fig. 2.

3.3. Calibration graph

The calibration dependence $t = f\ [c(I)]$ (where t stands for the ITP zone length in seconds and c(I) is the concentration of I in mg l^{-1}) was examined at the concentration range 15–180 mg l^{-1} of I and evaluated by linear regression. The calibration line $t = a \cdot c(I) + b$ is described by the following linear regression parameters: $a = 2.410 \pm 0.011$; $b = -0.16 \pm 1.17$; correlation coefficient $r = 0.99997$ (number of calibration points $n = 5$; each calibration solution was measured in triplicate).

The low value of the intercept and the high value of the correlation coefficient are positive signs of the correctly passed migration (analytical stability of the zone of I) and rectilinearity of the calibration curve, respectively. The results for replicate analyses of prepared sample of 60 mg l^{-1} of I ($n = 6$) had relative standard deviation (within-day) of 0.21% characterising good repeatability of the ITP assay. Day-to-day reproducibility of the calibration curves is characterised by the relative standard deviation of the slope and intercept data not exceeding 1.5%.

The LOD and LOQ values are 2 and 5 μg ml^{-1} of tramadol, respectively as calculated by statistical analysis of the calibration data.

3.4. Determination of I in pharmaceutical formulations

The ITP was used for determining I in seven pharmaceutical preparations. In all instances single-component tramadol preparations have been analysed since we could not find any commercial formulations involving combination of I with another active principle. The results of ITP assays are summarised in Table 2. Because of good solubility of I (hydrochloride) in water the sample preparation for analysis was straightforward (Section 2). The rate of liberation of I from the Tramal suppository sonicated at 40°C is depicted in Fig. 3; it is clearly seen that the minimum time of sonication needed for complete extraction of I from the suppository base is 10 min. Since standard pharmacopoeial methods for the assay of I are not available, we decided to check the accuracy of our ITP results by the method of the standard addition; the recoveries of the added I ranging between 97.2–100.1% are shown in Table 3. Hence it can be concluded that the accuracy of the proposed ITP assay of I in various dosage forms is acceptable.

Referring to the content of tramadol in dosage forms and to the precision data shown in Table 2 it is possible to use the described ITP procedure as a stability-indicating assay. There was no indication of instability of tramadol during the ITP assay.

4. Conclusions

The results presented in this paper indicate that the cationic capillary ITP is a suitable tool for separating and determining milligram amounts of analgesic-anodyne tramadol in various dosage forms. The method is acceptably time efficient; a single analysis takes < 20 min including the sample preparation. Compared to the published LC method [4] the proposed ITP assay is more friendly to environment (no organic solvents used), has similar sample throughput as the HPLC and its sensitivity, though lower than that of HPLC with fluorescence detection, is fully sufficient for the analysis of pharmaceutical preparations. On the other hand, the proposed ITP method cannot be utilised for determining low levels of tramadol in body fluids. The precision of the method, expressed as RSD, was 0.5–1.2% (six replicates) when analysing seven commercial preparations including capsules, drops, injections and suppositories. Therefore the ITP method can be recommended for analytical evaluation of pharmaceutical formulations containing tramadol as the active ingredient.

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