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Comparison of isotachophoresis, capillary zone electrophoresis and high-performance liquid chromatography for the determination of salbutamol, terbutaline sulphate and fenoterol hydrobromide in pharmaceutical dosage forms

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ABSTRACT

Reversed-phase high-performance liquid chromatography (RP-HPLC), isotachophoresis (ITP) and capillary zone electrophoresis (CZE) were applied to the determination of salbutamol, terbutaline sulphate and fenoterol hydrobromide in commercially available pharmaceutical dosage forms. The comparison showed that especially with the use of ITP, high concentrations of other charged sample components can disturb the separation process. If special attention is paid to ensure a complete separation, all methods give comparable results. For the regression lines of the calibration graphs, regression coefficients of at least *ca.* 0.999 and nearly zero intercepts are obtained with relative standard deviations of *ca.* 1–2% for peak area or zone lengths. By applying the different techniques, often different components of the sample matrix can be detected, *i.e.*, a more complete impression of the sample composition can be obtained by using all the three techniques.

INTRODUCTION

With the development of capillary zone electrophoresis (CZE), the analyst now has available several separation techniques, isotachophoresis (ITP), CZE and high-performance liquid chromatography (HPLC), with an overlap of application areas. Although ITP can be applied for the separation of uncharged components, *e.g.*, by complexation with charged additives in the electrolyte system, ITP is most suitable for the separation of charged components and the separation principle is based on differences in the effective mobilities of the components. The effective mobilities can be affected, *e.g.*, by changing the pH of the electrolyte system and the addition of complexing agents.

HPLC can be applied to both uncharged and charged components and the separation principle is based on partitioning between a stationary and a mobile phase. The capacity factors, k', can be affected by changing, *e.g.*, the polarity and pH of the mobile phase and the addition of complexing agents.

In CZE, the separation principle is based on differences in effective mobilities although the application of a second mobile phase leads to a hybrid technique by which the separation principle depends on both differences in effective mobilities and partitioning over two mobile phases (micellar electrokinetic capillary chromatography). In the latter instance uncharged components can also be separated.

Altogether, it is clear that the overlap in the application areas of ITP, CZE and HPLC lies in the separation of charged components.

Although HPLC is often used for analyses of drugs [1–6], less attention has been paid to the use of

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Salbutamol

Terbutaline

Fenoterol

Fig. 1. Structural formulae of salbutamol, terbutaline and fenoterol.

ITP and CZE and to compare these methods concerning reproducibility and accuracy. Particularly for CZE, few quantitative applications have been published.

In this paper, the abilities of ITP, CZE and HPLC are compared for the determination of some drugs in pharmaceutical dosage forms. As the UV detector of the Beckman CZE apparatus has standard wavelengths of 214, 254 and 280 nm, we selected for the quantitative comparison salbutamol, fenoterol and terbutaline because they have optimum UV absorbance at a wavelength of about 214 nm. For ITP the choice of the wavelength is of less importance because the sample zone concentration is high compared with CZE and HPLC. The structural formulae of the drugs are given in Fig. 1.

EXPERIMENTAL

Instrumentation

The HPLC equipment (Pharmacia–LKB, Bromma, Sweden) consisted of a Model 2150 pump, a Model 2152 controller, a low-pressure mixer, a Model 2156 solvent conditioner and a VWM 2141 dual-wavelength UV detector. Chromatographic separation was obtained with a LiChrospher 100 RP-18 end-capped column (125 × 4 mm, 5 μm) from E. Merck (Darmstadt, Germany). Injections were made with a Model 7125 universal loop injector (20 μl) (Rheodyne, Berkeley, CA, USA).

For all CZE experiments the P/ACE System 20 HPCE (Beckman, Palo Alto, CA, USA) was us The capillary was an original Beckman capilla cartridge (capillary length 57 cm, distance betwe injection and detection 50 cm and 75 μm I.D.). T wavelength of the UV detector was 214 nm in experiments and the operating temperature w 25°C. All experiments were carried out in t cationic mode (anode placed at the inlet and catho at the outlet), applying a constant voltage of 1 kV. The sample introduction was achieved pressure injection for 5 s (about 100 nl).

For all ITP experiments, a laboratory-built ap ratus [7] with conductivity and UV detectors (2 nm) was used. In this apparatus, a closed system obtained by shielding the separation capillary fr the open electrode compartments with semiper able membranes. A PTFE capillary tube (0.2 n I.D.) was used, in contrast to the fused-silica ca lary in the Beckman apparatus. The sample w introduced with a syringe and the sample volu was 3 μl, unless stated otherwise.

All data obtained from the chromatograms a electropherograms were handled using the labo tory-written program CAESAR.

Chemicals

Salbutamol sulphate, terbutaline sulphate a fenoterol hydrobromide were kindly donated by t State Institute of Quality Control for Agricultu

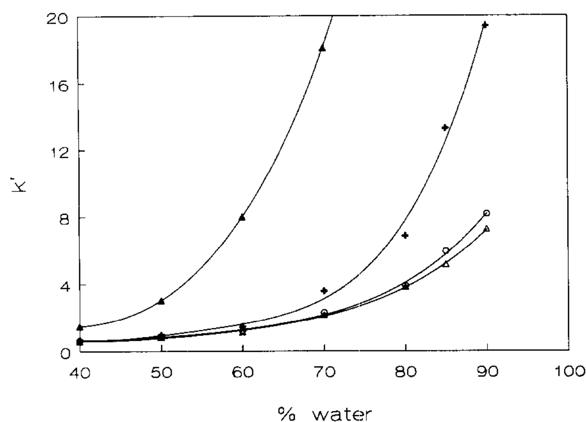

Fig. 2. Relationship between capacity factor, k', and percentage of water in the water–methanol mobile phase containing 0.002 *M* KOH and 0.01 *M* hexanoic acid for (△) terbutaline sulphate, (○) salbutamol sulphate, (+) fenoterol hydrobromide and (▲) the analogue antibiotic clenbuterol hydrochloride.

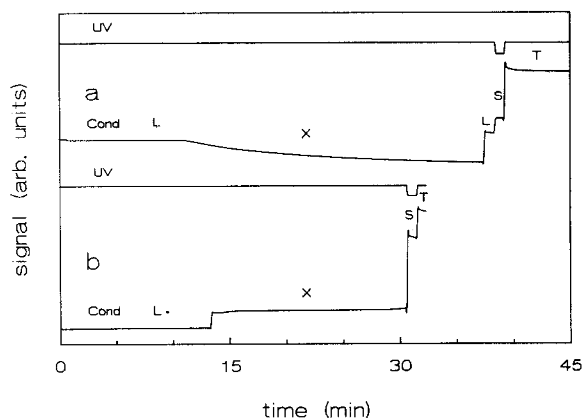

Fig. 3. Isotachopherograms for the analysis of Ventolin syrup by ITP applying (a) electrolyte system A and (b) electrolyte system B. The zone of salbutamol is indicated by S. The unknown sample component X migrates isotachophoretically in system B and zone electrophoretically in the leading zone histidine in system A. L = leading electrolyte, T = terminator.

Products (RIKILT, Wageningen, Netherlands). All salbutamol pharmaceuticals are Ventolin products from Glaxo (Nieuwegein, Netherlands), the terbutaline pharmaceuticals are Bricanyl products from Astra Pharmaceutica (Rijswijk, Netherlands) and the fenoterol pharmaceuticals are Berotec products from Boehringer Ingelheim (Alkmaar, Netherlands).

Standard solutions

Standard solutions of 1 mg/ml of salbutamol, terbutaline sulphate and fenoterol hydrobromide were prepared by weighing accurately 50.0 mg of the standards and dissolving them in 50.0 ml of distilled water. From these solutions appropriate dilutions were made so that the concentration of each sample solution approached the concentration of that in the middle of the standard solution range.

Sample preparation

All tablets and the capsules were mixed with 10 ml of water and, after ultrasonication for about 30 min, the sample solution was centrifuged. The clear supernatant solution was used for the analysis after dilution with distilled water to the desired concentration. All liquid pharmaceuticals were diluted to the desired concentration with distilled water.

Separation conditions for HPLC

Reversed-phase HPLC was performed at ambient temperature. Several experiments were carried out to select a suitable mobile phase and water–methanol (60:40, v/v) containing 0.002 *M* KOH and 0.01 *M* hexanoic acid as an ion-pair reagent was found suitable for the analysis of the pharmaceuticals. In Fig. 2 the capacity factor, k', is given as a function of the percentage of water in the water–methanol mixture for salbutamol sulphate, fenoterol hydrobromide, clenbuterol hydrochloride and terbutaline sulphate. The mobile phase was degassed by vacuum filtration through a 0.22-μm filter and sparging with helium. The column was equilibrated with mobile phase at a flow-rate of 0.4 ml/min for about 1 h.

Separation conditions for ITP

For the ITP experiments, two electrolyte systems were used. System A consisted of a leading electrolyte of 0.01 *M* histidine adjusted to pH 4.75 by adding acetic acid with the terminator acetic acid at pH 3.5. System B consisted of a leading electrolyte of 0.01 *M* KOH adjusted to pH 4.75 by adding acetic acid with the terminator acetic acid at pH 3.5.

With electrolyte system A, ionic species present in the sample solutions with high effective mobilities (such as sodium) will migrate in a zone electrophoretic manner through the leading zone of histidine.

TABLE I

AVERAGE VALUES (AV) AND RELATIVE STANDARD DEVIATIONS (R.S.D.) OF THE RELATIVE STEP HEIGHTS *RSH* (AS % OF THE STEP HEIGHT OF PYRAZOLE-3,5-DICARBOXYLIC ACID) AND ZONE LENGTHS *ZL* WITH ITP

Component	*RSH* (AV)	R.S.D. (%)	*ZL* (s) (AV)	R.S.D. (%)
Chloric acid	63.6	1.35	13.23	1.18
Malonic acid	83.6	0.54	24.23	0.90
Pyrazole-3,5-dicarboxylic acid	100.0	–	27.02	0.65
Acetic acid	141.6	0.71	15.93	1.57
Glutamic acid	237.1	1.31	19.58	0.62

The drugs migrate in an ITP manner between the leading ions histidine and the terminating hydrogen ions. Applying electrolyte system B, the drugs migrate behind a large zone of the sample ions with a high effective mobility. Nevertheless, identical results were obtained for test samples with both systems A and B. As an example, the isotachopherograms (both the UV and conductivity detector signals) of Ventolin syrup are given in Fig. 3, applying both (a) system A and (b) system B. It can clearly be seen that in system B, salbutamol migrates behind a large amount of a sample cation with high mobility, whereas in system A that sample cation migrates in the leading zone of histidine. For the determination of the drugs in the pharmaceuticals we applied system B.

Separation conditions for CZE

All CZE experiments were carried out with th background electrolyte 0.01 *M* tris(hydroxymethy aminomethane (Tris) adjusted to pH 5.0 by addin acetic acid.

RESULTS AND DISCUSSION

In the comparison of the efficiency of ITP, HPL and CZE for the determination of components i different samples, validation of the methods is a important task. The validation requires a demo stration of the specificity, sensitivity, calibratio linearity, precision and accuracy of the method For this reason we shall first consider the within-da precision (repeatability) and between-day precisio (reproducibility) and compare the calibratio graphs obtained with the different methods.

Within-day precision

In order to obtain an impression of the within-da precision of the methods, replicate separations (n = 10) were made of sample mixtures of five compo nents with the apparatus for ITP, HPLC and CZE

With the ITP apparatus we performed separa tions of a mixture of chloric, malonic, pyrazole-3,5 dicarboxylic, acetic and glutamic acids (all at concentration of $8 \cdot 10^{-4}$ *M*) applying a leadin electrolyte of 0.01 *M* HCl adjusted to pH 6.0 b adding histidine and a terminating electrolyte c 0.01 *M* 2-(N-morpholino)ethanesulphonic aci (MES). The current was 25 μA. In Table I th average values and relative standard deviations a given for the relative step heights as a percentage c

TABLE II

AVERAGE VALUES (AV) AND RELATIVE STANDARD DEVIATIONS (R.S.D.) OF THE RETENTION TIMES t_R AN PEAK AREA *A* WITH HPLC

Component	t_R (min) (AV)	R.S.D. (%)	*A* (AUs) (AV)	R.S.D. (%)
Toluene	3.58	0.11	5.47	0.92
Ethylbenzene	4.43	0.15	5.36	1.00
Propylbenzene	5.91	0.25	4.18	0.90
Butylbenzene	8.19	0.26	6.52	1.01
Pentylbenzene	11.66	0.34	7.15	1.42

TABLE III

AVERAGE VALUES (AV) AND RELATIVE STANDARD DEVIATIONS (R.S.D.) OF THE MIGRATION TIMES t_m, EFFECTIVE MOBILITIES m_{eff} AND PEAK AREA A WITH CZE

Component	t_m (min) (AV)	R.S.D. (%)	$m_{eff} \times 10^5$ ($cm^2/V \cdot s$) (AV)	R.S.D. (%)	A (mAUs) (AV)	R.S.D. (%)
Salbutamol	5.54	1.01	19.07	0.72	12.29	1.51
Creatinine	6.12	1.02	12.61	0.90	6.72	2.08
Aniline	6.43	1.05	9.56	1.15	26.37	1.21
m-Aminobenzoic acid	12.96	1.71	−20.20	0.95	21.29	0.88
Benzoic acid	17.19	2.19	−27.42	0.80	22.63	0.93

TABLE IV

BETWEEN-DAY PRECISION: AVERAGE VALUES OF PEAK AREA (A) MEASURED WITH HPLC AND CZE AND ZONE LENGTHS (ZL) MEASURED WITH ITP AND THE RELATIVE STANDARD DEVIATIONS (R.S.D.) FOR SEVERAL CONCENTRATIONS (c) OF SALBUTAMOL SULPHATE, WITH REGRESSION PARAMETERS r (REGRESSION COEFFICIENT), b (SLOPE) AND a (INTERCEPT)

c (mg/ml)	HPLC (n = 5)				CZE (n = 5)			
	Day 1		Day 2		Day 1		Day 2	
	A	R.S.D. (%)	A	R.S.D. (%)	A	R.S.D. (%)	A	R.S.D. (%)
0.100	24.08	0.46	24.89	0.19	59.43	0.18	58.36	6.21
0.075	18.75	0.09	18.58	0.08	45.78	0.58	44.15	0.54
0.066	16.51	0.13	16.44	0.11	39.44	0.20	39.06	1.14
0.050	12.39	0.14	12.28	0.13	29.38	1.09	30.16	1.55
0.033	8.24	0.14	8.23	0.06	19.33	0.17	19.90	0.29
0.025	6.13	0.04	6.16	0.09	14.27	0.28	14.48	0.20
0.010	2.51	0.02	2.51	0.01	5.72	0.04	5.60	0.15
r	0.99943		0.99996		0.99967		0.99976	
b	242.92		248.70		604.85		586.10	
a	0.20		−0.027		−0.54		0.11	
c (mg/ml)	ITP (conductivity) (n = 3)				ITP (UV) (n = 3)			
	Day 1		Day 2		Day 1		Day 2	
	ZL	R.S.D. (%)	ZL	R.S.D. (%)	ZL	R.S.D. (%)	ZL	R.S.D. (%)
1.000	124.93	0.66	126.37	0.34	124.60	1.00	126.60	0.47
0.750	94.15	0.97	95.42	1.05	94.00	0.98	95.80	1.30
0.625	76.43	0.69	77.52	1.10	76.20	0.79	78.20	1.17
0.500	64.22	0.72	64.55	0.60	64.60	1.07	63.63	0.95
0.375	46.95	0.32	46.62	0.54	46.20	1.30	46.60	1.49
0.250	31.33	0.24	31.40	0.55	31.60	1.10	31.40	1.10
0.100	12.65	0.40	12.85	1.56	12.38	1.22	12.37	0.71
r	0.99967		0.99970		0.99950		0.99988	
b	124.57		126.44		124.42		127.31	
a	0.32		−0.065		0.24		−0.53	

the step height of pyrazole-3,5-dicarboxylic acid and the zone lengths measured with the conductivity detector.

With the HPLC apparatus we analysed a mixture of toluene ($5.9 \cdot 10^{-5}$ *M*) and ethyl- ($5.1 \cdot 10^{-5}$ *M*), propyl- ($4.5 \cdot 10^{-5}$ *M*), butyl- ($8 \cdot 10^{-5}$ *M*) and pentylbenzene ($7.3 \cdot 10^{-5}$ *M*) in methanol, applying methanol–water (80:20) as eluent. The flow-rate was 1.0 ml/min. The UV detector wavelength was 209 nm. In Table II the average values and relative standard deviations (R.S.D.s) for the retention times and peak area are given.

With the CZE apparatus we analysed a mixture of salbutamol ($1.75 \cdot 10^{-5}$ *M*), creatinine ($2 \cdot 10^{-5}$ *M*), aniline ($1 \cdot 10^{-4}$ *M*), benzoic acid ($1 \cdot 10^{-5}$ *M*) and *m*-aminobenzoic acid ($2 \cdot 10^{-5}$ *M*), applying a background electrolyte of 0.01 *M* Tris at pH 5.0 adjusted by adding acetic acid. In Table III the average values and R.S.D.s are given for the migration times, calculated effective mobilities [8] and peak area.

The within-day precision for the techniques is about 1–2%.

Between-day precision

To establish the between-day precision we measured the peak area ($n = 5$) for CZE and HPLC and zone lengths ($n = 3$) for ITP (with both the conductivity and UV detectors) of salbutamol sulphate and calculated the average values and R.S.D.s for several different concentrations of the solute. This series was repeated with freshly prepared electrolyte solutions after 1 week. The results are given in Table IV. It can be concluded that the reproducibility of the HPLC experiments is by far the best. The high R.S.D. of 6.21% in the second series of CZE experiments is due to one bad value, which could not be considered statistically as an outlier, however. The ITP experiments were carried out only three times in order to be able to measure the complete calibration graph in 1 day.

Quantification and limit of detection

For the "limit of quantification" or "limit of determination", which can be regarded as the lower limit for precise quantitative measurements, we used the value $y_B + 3 s_B$, whereby the calculated intercept of the regression line can be used as an estimate of y_B and s_B is the standard deviation in the *y*-direction of the regression line [9].

The standard deviation in the concentration of unknown samples, determined with a calibration graph, is calculated according to the equation [9]

$$s = \frac{s_B}{b}\sqrt{\frac{1}{m} + \frac{1}{n} + \frac{(y - \bar{y})^2}{b^2 \sum_i (x_i - \bar{x})^2}} \quad (1)$$

where m is the number of measurements of the unknown sample, n is the number of points of the calibration graph, b is the slope of the calibration graph, $\bar{x}$ and $\bar{y}$ are the average values of the x and y values of the calibration points, x_i is the x value of the calibration points and y is the average value of the m measurements of the unknown sample.

Although in most papers the R.S.D. values of the results obtained with calibration graphs are calculated with the equation

$$s = \sqrt{\frac{\sum_i (x_i - \bar{x})^2}{n - 1}} \quad (2)$$

where x_i is the determined sample concentration and n is the number of measurements of the unknown sample, we prefer to use eqn. 1 because the "quality" of the calibration graph is included in the R.S.D. values. For techniques with a high precision, eqn. 2 leads to very low R.S.D. values, often unjustly indicating a high accuracy. The disadvantage of the use of eqn. 1 is that high R.S.D. values result if the sample concentration does not lie near the centroid of the regression line. For all quantitative determinations we applied the unweighted regression lines with seven concentrations per decade, taking care that the sample concentration lay near the centroid of the regression line.

Comparison of ITP, HPLC and CZE

For a first comparison of the separation methods we measured the peak area and zone lengths for samples of salbutamol sulphate from 1 to 0.001 mg/ml for HPLC and CZE and to 0.01 mg/ml for ITP and compared the linear regression lines. All zone lengths and peak area were recalculated as percentages of the highest values for each method. In Fig. 4A, B, C and D the values are presented graphically (logarithmic scale) for HPLC *versus* ITP (conductivity), CZE *versus* ITP (conductivity), CZE *versus* HPLC and conductivity *versus* UV detector

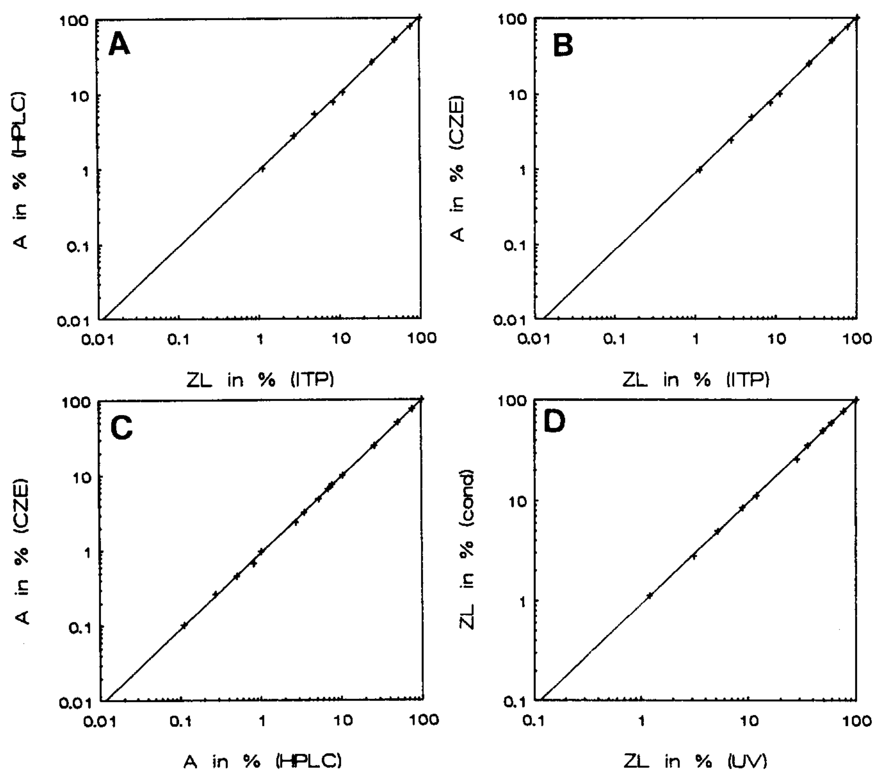

Fig. 4. Regression lines for the measured peak area A and zone lengths ZL as percentages of the maximum values for (A) HPLC *versus* ITP (conductivity signal), (B) CZE *versus* ITP (conductivity signal), (C) CZE *versus* HPLC and (D) conductivity *versus* UV signal for ITP.

signal for ITP, respectively. In Table V the slope, intercept, regression coefficient and limit of detection calculated for the regression lines (non-logarithmic scale) are given. The obtained linear relationships, with a slope nearly 1 and a nearly zero intercept, validate the techniques.

TABLE V

SLOPE, INTERCEPT, REGRESSION COEFFICIENT AND LIMIT OF DETECTION FOR THE INDIVIDUAL REGRESSION LINES OF PEAK AREA AND ZONE LENGTHS FOR SALBUTAMOL SULPHATE MEASURED WITH ITP, HPLC AND CZE AND CORRELATIONS BETWEEN THEM

Regression line of	Slope	Intercept	Regression coefficient	Detection limit (μg/ml)
ITP (conductivity)	139.21	0.1298	0.998929	41.20
ITP (UV)	115.45	0.8537	0.998508	58.38
CZE	744.97	−0.8805	0.999972	6.05
HPLC	283.72	0.3685	0.999960	7.17
HPLC–ITP (conductivity)	1.004	−0.096	0.999872	
CZE–ITP (conductivity)	0.999	−0.592	0.999804	
CZE–HPLC	0.991	−0.296	0.999871	
ITP (conductivity)–ITP (UV)	1.005	−0.604	0.999759	

Matrix effects

The composition of the sample can strongly influence the quality of the separation. Especially sample components with high effective mobilities present at a high concentration in a sample will affect the migration behaviour in electrophoresis. In CZE this can create an ITP system with two leading ions [10,11], leading to very sharp peaks and high plate numbers (this effect must be distinguished from stacking effects due to the injection of very dilute samples).

A typical difference between electrophoretic and chromatographic techniques is that in electrophoresis at any point the situation is determined by the initial conditions, as Kohlrausch formulated with his "regulation function" in 1897. This means that in electrophoresis the concentration of the injected sample adapts to the initial concentration of the background electrolyte migrating in the separation capillary. If one of the sample components is present at a high concentration, the length of the injected sample zone elongates during this adaptation process and the separation capacity of the system can be insufficient to separate all sample components. A way to solve this problem is to inject smaller amounts of the sample. However, the amount of the sample component of interest must be sufficient in order to detect and quantify that component. This is often a disadvantage in ITP, because the sample components migrate in consecutive zones, after the separation process, at a concentration adapted to that of the leading ions, which generally means at a concentration of about 0.005–0.01 *M*. Very small amounts of a sample component lead to very short, undetectable zones.

In ITP, the response factor, RF [12], defined as the slope of the calibration graph of the product $ZL \cdot I$ (A s) *versus* the amount of the sample Q (mol), can be utilized for quantitative determinations:

$$RF = \frac{ZL \cdot I}{Q} \tag{3}$$

where ZL is the zone length (s) and I the electric current (A). This RF value is a constant (about $2 \cdot 10^5$ C/mol for monovalent ions) and indicates that the minimum detectable amount can be decreased by applying lower values of the electric current, assuming that a minimum zone length is required. Of course, this results in longer analysis times. This principle was applied in the determination of salbutamol, fenoterol hydrobromide and terbutaline sulphate with ITP (see Tables VI–VIII). In first instance, deviating values were obtained. After diluting the sample solutions tenfold, injecting 1 μl of sample solution and applying 7 μA instead of 25 μA, good results were obtained for the previously too low values. Another way to solve this problem is to use a column-coupling system with a higher separation capacity.

Determination of drugs in pharmaceuticals

In Tables VI–VIII all results for the determination of salbutamol, terbutaline sulphate and fenoterol hydrobromide are given. For the calibration graphs, the concentration decade (CD), indicated with its highest concentration, the regression coefficient (r) and calculated limit of detection (LOD, μg/ml) are given. For all pharmaceuticals the number of measurements (m), the determined amount of the drugs (Q) and the calculated R.S.D. according to eqn. 1 are given.

For HPLC and CZE we used standard solutions in the decade 0.01–0.1 mg/ml, determined the calibration graph twice at a wavelength of 214 nm and applied each calibration graph for the quantification of all sample solutions ($m = 3$).

For ITP we worked in the first instance with standard solutions with concentrations in the concentration decade 0.1–1 mg/ml and applied a current of 25 μA. With the calibration graph, both using the conductivity and UV detector signals (injection volume 3 μl) we determined the amount of the drugs in all sample solutions and observed too low values for the liquid samples containing a very large amount of a sample component with a high effective mobility (probably sodium). After diluting these samples tenfold and working in the concentration decade 0.01–0.1 mg/ml (current 7 μA), the results covered the labelled values. In some instances (marked with asterisks in the tables) even 1 μl had to be injected in order to obtain a complete separation and in these instances we calculated the R.S.D. with the three lowest points ($n = 3$) of the concentration decade in order to obtain the measured zone length in the centroid of the regression line.

For the peak area in CZE we determined the temporal and not the spatial peak area because the

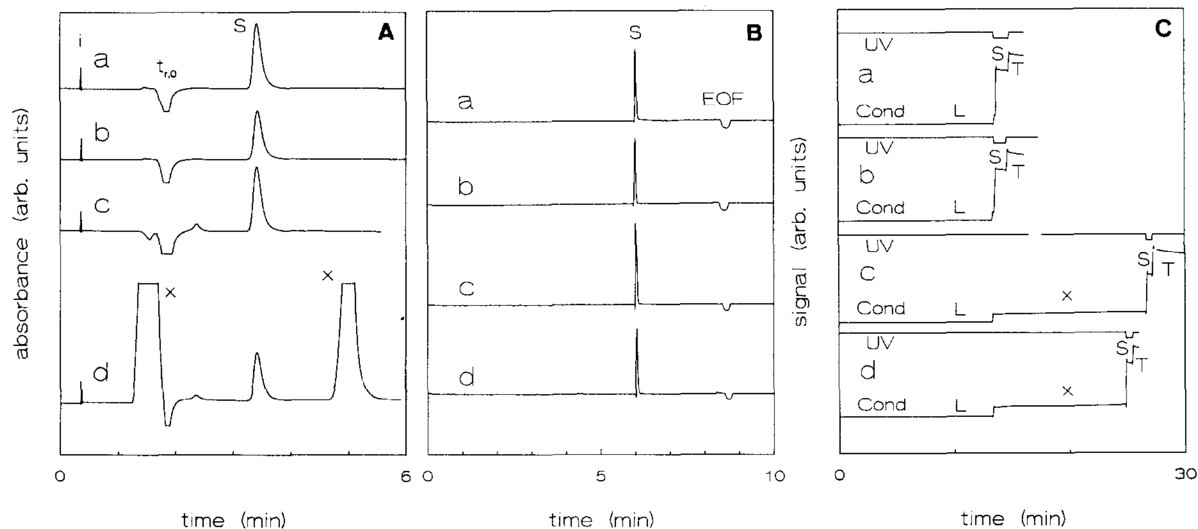

Fig. 5. (A) High-performance liquid chromatograms, (B) zone electropherograms and (C) isotachopherograms of (a) a standard solution of salbutamol, (b) the sample solution of Ventolin tablet, (c) the sample solution of the Ventolin solution for intervenous infusion and (d) the sample solution of Ventolin syrup. Salbutamol is indicated with S and all unknown components with X. In the isotachopherograms c and d the electric current is decreased to 7 μA at the time of detection of the zone X.

migration time in all experiments was fairly constant.

Determination of salbutamol

We studied the determination of salbutamol in Ventolin tablets (labelled value 4 mg per tablet), Ventolin solution for intravenous infusion (labelled value 1 mg/ml) and Ventolin syrup (labelled value 0.4 mg/ml). All the results are given in Table VI.

In Fig. 5, examples are given of the results of (A) HPLC, (B) CZE and (C) ITP experiments on (a) a standard solution of salbutamol, (b) a sample solution prepared from the Ventolin tablet, (c) the Ventolin solution for intravenous infusion and (d) the Ventolin syrup. For the HPLC and CZE experiments the UV signal and for the ITP experiments both the conductivity and UV signals are given. The salbutamol zones are marked with S. All other unknown sample components are indicated by X.

Comparing the chromatograms and electropherograms in Fig. 5A, B and C, some comments can be made. In all instances the salbutamol could be easily separated from other sample components without any pretreatment and the values obtained cover the labelled values, although for ITP a low current density had to be applied to obtain a complete separation from the matrix with sufficiently large zone lengths of salbutamol. For the Ventolin tablet, all the techniques show only the salbutamol component. For the Ventolin solution for intravenous infusion there is present at least one extra non-UV-absorbing sample component with a high effective mobility (probably sodium; see Fig. 5C), which could be present at about $t_{R,0}$ in the HPLC trace and is invisible in CZE. For the Ventolin syrup CZE shows only the salbutamol peak, ITP shows one non-UV-absorbing component and HPLC two extra UV-absorbing components. The time of analysis for HPLC and CZE is about 6 and 9 min [until electroosmotic flow (EOF) marker], respectively, and increases to about 30 min for the ITP analyses with samples containing a large amount of an unknown component X with a high effective mobility. An advantage of the electrophoretic methods is that the analysis can be stopped after the detection of the desired sample component, after which a new experiment can be started. A disadvantage of the HPLC method is the long equilibration time of the system and the fact that all components, including those not of interest, must pass the detector before a new run can be started.

In order to obtain more information about the

TABLE VI

AMOUNTS OF SALBUTAMOL (Q) IN VENTOLIN TABLETS, VENTOLIN SOLUTION FOR INTRAVENOUS INFUSION AND VENTOLIN SYRUP AND CALCULATED RELATIVE STANDARD DEVIATIONS (R.S.D.) DETERMINED WITH HPLC, CZE AND ITP

Method	Calibration graph			Tablet (4 mg per tablet)			Infusion (1 mg/ml)			Syrup (0.4 mg/ml)		
	CD	r	LOD	m	Q (mg per tablet)	R.S.D. (%)	m	Q (mg/ml)	R.S.D. (%)	m	Q (mg/ml)	R.S.D. (%)
HPLC												
214 nm	0.1	0.99985	1.76	3	4.05	1.02	3	1.04	0.81	3	0.40	1.03
214 nm	0.1	0.99977	1.80	3	4.00	1.04	3	1.02	0.83	3	0.40	1.03
CZE												
214 nm	0.1	0.99945	2.23	3	3.94	1.35	3	0.99	1.16	3	0.41	1.31
214 nm	0.1	0.99947	2.73	3	3.72	1.70	3	1.00	1.31	3	0.39	1.61
ITP												
UV	1	0.99933	37.0	3	3.96	2.20	3	0.90	3.06	3	0.34	2.62
Conductivity	1	0.99939	35.1	3	3.95	2.10	3	0.89	2.94	3	0.35	2.47
Conductivity	0.1	0.99931	3.12	3	4.07	1.77	3	1.00	1.91*	3	0.40	2.52*

TABLE VII

AMOUNTS OF TERBUTALINE SULPHATE (Q) IN BRICANYL TABLETS, BRICANYL AMPOULES FOR INJECTION AND BRICANYL SYRUP AND CALCULATED RELATIVE STANDARD DEVIATIONS (R.S.D.) DETERMINED WITH HPLC, CZE AND ITP

Method	Calibration graph			Tablet (5 mg per tablet)			Ampoules (0.5 mg/ml)			Syrup (0.3 mg/ml)		
	CD	r	LOD	m	Q (mg per tablet)	R.S.D. (%)	m	Q (mg/ml)	R.S.D. (%)	m	Q (mg/ml)	R.S.D. (%)
HPLC												
214 nm	0.1	0.99995	1.03	3	4.92	0.48	3	0.51	0.46	3	0.31	0.83
214 nm	0.1	0.99974	2.32	3	4.89	1.09	3	0.51	1.05	3	0.30	1.91
CZE												
214 nm	0.1	0.99895	4.62	3	4.68	2.28	3	0.52	2.05	3	0.30	3.89
214 nm	0.1	0.99952	3.11	3	4.67	1.54	3	0.50	1.43	3	0.30	2.62
ITP												

TABLE VIII

AMOUNTS OF FENOTEROL HYDROBROMIDE (Q) IN BEROTEC TABLETS, BEROTEC ROTACAPS AND BEROTEC RESPIRATOR SOLUTION AND CALCULATED RELATIVE STANDARD DEVIATIONS (R.S.D.) DETERMINED WITH HPLC, CZE AND ITP

Method	Calibration graph			Tablet (2.5 mg per tablet)			Rotacaps (0.2 mg per capsule)			Respirator (5 mg/ml)		
	CD	r	LOD	m	Q (mg fenoterol per tablet)	R.S.D. (%)	m	Q (mg fenoterol hydrobromide per capsule)	R.S.D. (%)	m	Q (mg/ml fenoterol hydrobromide)	R.S.D. (%)
HPLC												
214 nm	0.1	0.99983	1.54	3	1.84	0.76	3	0.123	2.27	3	4.98	0.71
214 nm	0.1	0.99982	1.92	3	1.86	0.94	3	0.125	2.78	3	4.99	0.89
CZE												
214 nm	0.1	0.99986	1.70	3	1.88	0.82	3	0.121	2.57	3	4.89	0.80
214 nm	0.1	0.99935	3.64	3	1.97	1.68	3	0.117	5.73	3	4.91	1.71
ITP												
UV	1	0.99985	17.53	3	1.91	0.84	3	0.133	1.68	3	4.14	0.99
Conductivity	1	0.99994	10.94	3	1.89	0.53	3	0.136	1.02	3	4.24	0.60
Conductivity	0.1	0.99995	1.05	3	1.83	0.52	3	0.154	1.19	3	4.96	0.49

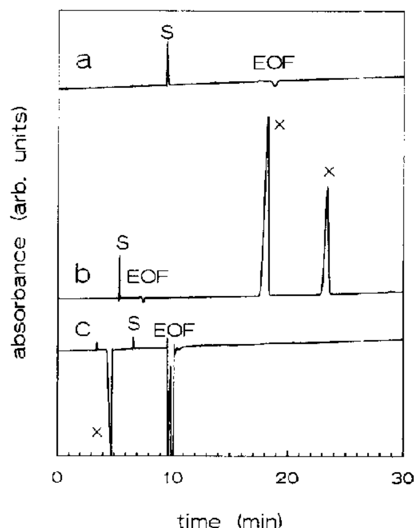

Fig. 6. Zone electropherograms of Ventolin syrup applying as background electrolyte (a) Tris–acetate (pH 5.0), (b) Tris–acetate (pH 5.0) after rinsing the capillary tube with 0.1 *M* KOH to obtain a higher velocity of the EOF and (c) histidine–acetate (pH 5.0). For further explanation, see text.

sample composition of Ventolin syrup, we performed an experiment with the background Tris–acetate at pH 5.0 (see Fig. 6a, where only one sample component salbutamol could be observed). After carefully rinsing the capillary tube with 0.1 *M* KOH, in order to obtain a higher velocity of the EOF, we repeated this separation (see Fig. 6b). With the high EOF also negative ions can be detected and two UV-absorbing negative ions are present in the electropherogram. We repeated this separation with the background electrolyte 0.01 *M* histidine adjusted to pH 5.0 by adding acetic acid. Non-UV-absorbing positive ions can now be made visible by indirect UV detection and in Fig. 6c it can clearly be seen that also a non-UV-absorbing positive ion with a high effective mobility is present in the sample solution.

Applying a background electrolyte with UV-absorbing ions affects the UV signal of salbutamol [13], as can be observed in Fig. 6c. Summarizing, the results indicate that the sample of Ventolin syrup contains at least four components, *viz*., salbutamol, a non-UV-absorbing positively charged component and two UV-absorbing negatively charged components. We repeated the CZE experiments with several different background electrolytes at pH values from 4 to 8 and obtained good separation showing that the choice of the background electr lyte is not critical.

Determination of terbutaline sulphate

For the determination of terbutaline sulphate v used Bricanyl tablets (labelled value 5 mg per tablet Bricanyl ampoules for injection (labelled value 0 mg/ml) and Bricanyl syrup (labelled value 0 mg/ml). All results for terbutaline sulphate are give in Table VII.

As for salbutamol, terbutaline could easily l separated from all other sample components witl out any pretreatment. The values obtained for tl Bricanyl tablet with CZE are slightly lower th those for the other methods. The liquid Brican samples also contain some other components, vis ble with HPLC and ITP.

Determination of fenoterol hydrobromide

Fenoterol hydrobromide was determined Berotec tablets (labelled value 2.5 mg of fenoter per tablet; all measured values were recalculated fenoterol per tablet), Berotec Rotacaps (labell value 200 μg of fenoterol hydrobromide per capsul and Berotec respirator solution (labelled value 5 n of fenoterol hydrobromide/ml). All results are give in Table VIII.

The results of the respirator solution cover the labelled values. All methods show comparab values for the amount of fenoterol per tabl although much lower than the labelled value. In tl sample preparation, much of the tablet did n dissolve and probably fenoterol partially adsorbs the insoluble components. In the comparison of tl techniques we did not seek a sample preparati with 100% recovery.

A similar problem occurred with Berotec Rot caps. In the isotachopherograms a slow UV-a sorbing component could be observed migrati between fenoterol and the terminating hydrog zone, probably in an enforced way [14,15]. Th component is partially mixed with fenoterol. F this reason, the determined values are higher th those of HPLC and CZE. On diluting the samp solution, the determined amount of fenoterol i creases because fenoterol is completely separat from the unknown sample component with hi effective mobility and the zone is enlarged owing

the presence of the unknown UV-absorbing sample component with low effective mobility.

CONCLUSIONS

In the analyses with ITP, CZE and HPLC for all components, a linear relationship between measured peak area or zone length and concentration of the components is obtained with regression coefficients better than about 0.999 and R.S.D. values up to about 2%. ITP and CZE seem to be more sensitive to irreproducibilities in, amongst others, the injected amounts, through which the repeatability of the HPLC values seem to be slightly better than those of ITP and CZE, although a disadvantage of the HPLC technique is the decreasing column quality.

In the comparison of the measured peak areas and zone lengths for the different techniques, regression lines were obtained with slopes of about 1 and nearly zero intercepts, validating these techniques. Application of the techniques to the determination of salbutamol, terbutaline sulphate and fenoterol hydrobromide in several pharmaceutical dosage forms gave comparable results covering the labelled values although, especially in the electrophoretic techniques, other sample components, present at high concentrations, can affect the separation. In CZE with high EOF, both anions and cations can be observed, in contrast to ITP. For most pharmaceuticals a very simple pretreatment is sufficient to obtain sample solutions. This procedure is, however, not adequate to desorb fenoterol from the Berotec tablets and Rotacaps. Because the aim of this investigation was to compare ITP, CZE and HPLC we did not seek a procedure to desorb the fenoterol completely. The combined application of these techniques provides more information about the sample composition.

In conclusion, it can be stated that CZE can compete with well established techniques such as HPLC and ITP for the determination of drugs in fairly simple matrices with regard to time of analysis and quantification, whereas the choice of the background electrolyte is not critical.

REFERENCES

1 M. J. Hutchings, J. D. Paull and D. J. Morgan, *J. Chromatogr.*, 277 (1983) 423.
2 N. Kurosawa, S. Morishima, E. Owada and K. Ito, *J. Chromatogr.*, 305 (1984) 485.
3 V. Das Gupta, *J. Liq. Chromatogr.*, 9 (1986) 1065.
4 S. Ray and A. Bandyopadhyay, *Indian Drugs*, 27 (1990) 313.
5 J. E. Kountourellis and C. Markopoulou, *J. Liq. Chromatogr.*, 12 (1989) 3279.
6 D. A. Williams, E. Y. Y. Fung and D. W. Newton, *J. Pharm. Sci.*, 78 (1982) 956.
7 F. M. Everaerts, J. L. Beckers and Th. P. E. M. Verheggen, *Isotachophoresis, Theory, Instrumentation and Applications*, Elsevier, Amsterdam, 1976.
8 J. L. Beckers, F. M. Everaerts and M. T. Ackermans, *J. Chromatogr.*, 537 (1991) 407.
9 J. C. Miller and J. N. Miller, *Statistics for Analytical Chemistry*, Ellis Horwood, Chichester, 1984.
10 J. L. Beckers and F. M. Everaerts, *J. Chromatogr.*, 508 (1990) 3.
11 J. L. Beckers and F. M. Everaerts, *J. Chromatogr.*, 508 (1990) 19.
12 J. L. Beckers and F. M. Everaerts, *J. Chromatogr.*, 470 (1989) 277.
13 M. T. Ackermans, F. M. Everaerts and J. L. Beckers, *J. Chromatogr.*, 549 (1991) 345.
14 P. Gebauer and P. Boček, *J. Chromatogr.*, 267 (1983) 49.
15 P. Boček and P. Gebauer, *Electrophoresis*, 5 (1984) 338.