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Development and validation of a capillary electrophoresis–indirect photometric detection method for the determination of the non-UV-absorbing 1,4-dideoxy-1,4-imino-D-arabinitol in active pharmaceutical ingredients, solutions and tablets using an internal standard

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Abstract

A high speed, selective, and robust capillary electrophoresis (CE) method with high capacity was developed and validated for determination of assay of 1,4-dideoxy-1,4-imino-D-arabinitol in active pharmaceutical ingredients, solutions, and tablets during the development work at preclinical and Phase I and II clinical studies. 1,4-Dideoxy-1,4-imino-D-arabinitol, tartrate has (almost) no UV absorption. Therefore, the developed CE method for quantification was based on indirect UV detection. A cation CE principle was chosen using an electrolyte at pH 4.0 containing dimethyldiphenylphosphonium hydroxide, which has a strong UV absorbance. The quantification was performed using internal standard technique, by which piperidine was used as internal standard. The method was validated. The validation results showed that the CE method was suitable for the assay (and dissolution) analysis.

Keywords: Pharmaceutical analysis; Indirect detection; Validation; Dideoxyiminoarabinitol; Piperidine

1. Introduction

Indirect detection methods are generally used in capillary electrophoresis when direct detection technique such as UV and fluorometric are not usable. Indirect photometric detection in capillary electrophoresis is valuable as a detection method for non-UV-absorbing low-molecular mass ions [1–4]. The general operating principle is based on displacement of an UV absorbing ion added to the electrolyte by analyte co-ion, which are ions of the same charge polarity, as they migrate under the influence of the electric field through the capillary.

1,4-Dideoxy-1,4-imino-D-arabinitol (DAB), tartrate is a pyrrolidine type drug and a candidate for treatment of Type 2 diabetes. The compound has been developed as a glycogen phosphorylase inhibitor (GPI) compound [5–7]. The structure of 1,4-

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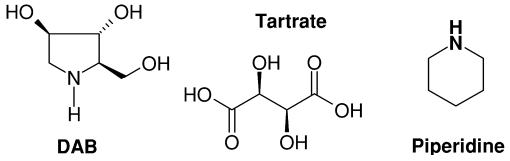

Fig. 1. Structure of 1,4-dideoxy-1,4-imino-D-arabinitol, tartrate also known as DAB, (−) tartrate and structure of piperidine used as internal standard in the CE method for quantification of DAB.

dideoxy-1,4-imino-D-arabinitol, (−) tartrate is given in Fig. 1.

DAB, (−) tartrate has (almost) no UV absorption, therefore, a traditional HPLC method with UV or fluorometric detection was not suitable for analytical tests. Other detection techniques such as mass spectrometry (LC–MS), refractive index, and electrochemical (both with HPLC) were considered. CE–indirect UV detection was evaluated to be a good choice, as it could also easily be used for quantification analysis. Therefore, a CE method for assay and dissolution determination analysis was developed based on indirect UV detection using an electrolyte at pH 4.0 containing dimethyldiphenylphosphonium (DDP) hydroxide, which has a strong UV absorbance.

The quantification was performed using internal standard technique, by which piperidine was used as internal standard. As part of the documentation, the method was validated according to official guidelines [8,9].

Validation was performed with respect to specificity (selectivity), linearity, range, accuracy, repeatability, intermediate precision, limit of detection (LOD), limit of quantification (LOQ), robustness, and stability of standard and sample solutions. The validation results showed that the CE method was suitable for the quantitation analysis (assay and dissolution).

2. Experimental

2.1. Chemicals, materials, reagents, and solutions

Deionized water was obtained from a Milli-Q system, piperidine, (99%; internal standard=ISTD, density 0.86 g/ml), from Aldrich, dimethyldiphenylphosphonium iodide (purum, II grade; DDP-I) from Fluka, α-hydroxyisobutric acid (puriss grade HIBA), from Fluka, 1.0 *M* sodium hydroxide (diluted sodium hydroxide, e.g., Titrisol 1.09956) from Merck, OnGuard A ion-exchange cartridge (P/N 42102; an anionic resin) from Dionex, 0.45 μm polypropylene filter from Cameo and DAB, D-(−)-tartrate standard, DAB, D-(−)-tartrate related substances as standards, active pharmaceutical ingredients (API) and tablets from Novo Nordisk (Maaloev, Denmark).

2.1.1. Preparation of solutions

The procedure for making the running electrolyte was taken from a Dionex Application [10].

DDP-I, 25 m*M*: 428 mg was dissolved in 50 ml of deionized water. Since the salt was not instantly dissolved, sonication was used to speed up the process. DDP iodide was not soluble in water at concentrations above 25 m*M*. The storage time of the solution was 3 months in refrigerator.

HIBA, 100 m*M*: 520 mg was dissolved in 50 ml of deionized water. The storage time of the solution was 3 months in refrigerator.

Dimethyldiphenylphosphonium hydroxide (DDP-OH), 25 m*M*: The 25 m*M* DDP iodide solution was converted to the hydroxide form by using an anion exchange resin in the hydroxide form. OnGuard A cartridge filter was prepared by passing 10 ml 1.0 *M* NaOH through it followed by a wash with deionized water (10 ml) to bring the pH below 7. Having converted the resin to the hydroxide form, 10 ml DDP-I was passed through the cartridge. The first 2 ml were discarded, and only the final 8 ml were collected. If more than 8 ml of DDP-OH was required, several cartridges were used, since they were single use cartridges. The storage time of the solution was 1 month in refrigerator.

Preparation of the electrolyte (for electrophoresis): to make 100 ml of the electrolyte, 20.00 ml 25 m*M* DDP-OH was added 6.00 ml of 100 m*M* HIBA in a 100-ml volumetric flask. The pH was adjusted to 4.00 with 100 m*M* HIBA, and the solution was brought to the volume with deionized water. The electrolyte was then filtered using a 0.45-μm pore filter. The storage time of the electrolyte was 1 month in refrigerator.

2.2. Instrumentation

Capillary electrophoresis was performed using an Agilent Technologies ^{3D}CE system (Agilent Technologies). Data acquisition and signal processing were preformed using Agilent Technologies ^{3D}CE ChemStation (rev. A.06.03, Agilent Technologies).

The instrumentation is specified in Table 1.

2.3. Quality controls and standard solutions

Internal standard (ISTD) solution (64.5 μg piperidine/ml) was added to all quantification samples. The internal standard was prepared as follow: in a 1000-ml volumetric flask, 75 μl piperidine standard was added to 100 ml deionized water, and it was brought to volume with deionized water. The storage time of the electrolyte was 1 week in a refrigerator.

Same internal standard stock solution was used for adding the internal standard to DAB standard solution, control solution, and samples.

Stock standard solution (987 μg DAB as free base/ml): in a 10-ml volumetric flask, 21.0 mg DAB, (−) tartrate standard were dissolved in 5 ml deionized water and brought to volume with deionized water. The storage time of the stock solution was 1 month in refrigerator.

Standard solution (39.5 μg DAB as free base/ml) for quantification: 1000 μl DAB stock standard solution were transferred to a 25-ml volumetric flask, and it was brought to volume with ISTD solution. The storage time of the stock solution was 1 week in a refrigerator.

Control solution (39.5 μg DAB as free base/ml) for checking the recovery of the method was prepared as stock standard solution and standard solution (identical).

The exact concentration (μg/ml) of DAB (as free base) in standard solution and control solution was calculated as:

$$C_{\text{DAB (base)}} = \frac{\text{mg [DAB, (−) tartrate standard]} \times 0.4701^{*} \times 1000 \times 1}{10 \times 25}$$

*(all sample concentrations, stock solutions and dilutions were given in mass units as free base of DAB. One mol of DAB, (−) tartrate weight 283.24 g/mol and 1 mol of DAB as free base mass 133.15 g/mol).

Table 1
Parameters for the CE indirect detection method for determination of assay (dissolution) of DAB, (−) tartrate in API, solutions, and tablets

Capillary	48.5 cm (40 cm efficient length)×50-μm I.D. ''Extended Light Path Capillary'', e.g., Agilent Technologies capillary
Detection	UV-detection 215 nm as sample cell: 350 nm (band width 80 nm) and reference cell: 215 nm (band width 16 nm). This change is to detect the DAB peak as a ''normal'' peak (and not upside down)
Autosampler temperature	Room/autosampler temperature (approx. 21 °C)
Electrolyte	DDP-OH (5 m*M*) pH 4.0
Preconditioning	30 min with the electrolyte
Rinse	After each sample (if needed), the capillary was cleaned with water and then electrolyte (flush for 1 min with each) Cleaning the capillary after runs (if needed): was performed with 0.1 *M* NaOH and water (flush for 5 min with each)
Injection	Hydrodynamic injection Quantification analyses (assay and dissolution samples): 20 mbar for 5.0 s (approximately 2 nl) followed by 5 s at 20 mbar injection of the electrolyte
Voltage	+30 kV (the polarity is normal, the detector side is the cathode)
Ampere	50 μA (approx.)
Capillary temperature	30 °C
Run time	Assay: 3 min

2.4. Sample preparation

The analysis concentrations for sample solutions (assay and dissolution) and standard solution were approximately 40 μg/ml for DAB and 30 μg/ml for piperidine as internal standard. The samples were prepared as follow:

API sample solution were prepared and diluted as the standard solution (see Section 2.3). Dosing solutions were with following concentration: 1.00, 2.00, 4.00, 4.50, 5.00, 10.0, 20.0, 40.0, 47.0, and 50.0 mg/ml of DAB (as free base). The sample solution: dosing solution were diluted with water to concentration 1000 μg/ml and then were prepared and diluted as the standard solution (see Section 2.3). Sample solution for tablets: Tablets of 1.00, 12.5, 25, 50, 100, 125, 200, and 300 mg DAB (as free base) were extracted in water to a concentration of 1000 μg/ml for DAB (as free base). The extraction was performed by adding the tablets to fixed volume water. This mixture was stirred for approximately 60 min and centrifuged at 4000 rpm for 10 min. Then, the sample was added internal standard and diluted (as the standard solution) (see Section 2.3). Placebo tablets were prepared as 1.00-mg tablets (highest amount of pharmaceutical excipient).

Dissolution samples: the dissolution test was a paddle method (USP/Ph.Eur., apparatus 2) in 1000 ml 37 °C 0.01 N hydrochloride acid (pH 2.0), rotation speed was 75 rpm with sampling at 15 and 30 min. The dissolution test was only performed on 50-, 100-, 200-, and 300-mg tablets. The test was performed on one tablet in 1000 ml of dissolution test media. A sample of 10 ml was taken and diluted as shown in Table 2.

2.5. Method conditions

The CE method is described in Table 1. This method was designed to provide analytical data for quantification/assay of DAB (as free base) in drug substance (API), dosing solutions, tablets, and dissolution samples.

For these purposes, the following points were considered for validation: specificity (selectivity), linearity, range, accuracy, repeatability, intermediate precision, LOD, LOQ, robustness, and stability of solutions.

3. Results and discussion

3.1. Development of the CE method (short background)

The first target of method development was detection of the non-UV-absorbing compound DAB. Possible detection methods and analytical techniques were considered. The CE seemed to be a very good choice, as it could be used for quantification analysis. The use of indirect UV detection in CE has also been shown to work and widely used in published CE methods [1–4].

A cation indirect detection at low pH was chosen. A number of cationic UV-absorbing substances were tested to provide the background UV signal for indirect UV detection. After test of different combinations the most stable system was chosen.

The best detection and separation was achieved using a Dionex cation CE analysis method [10]. The system was based on indirect UV detection using an

Table 2
Dilution of dissolution samples for quantification of DAB

Tablet (mg)	Diluting factor	Diluted as v/v ml with ISTD	End concentration of DAB as μg free base/ml in analyzed samples	End concentration of ISTD as μg/ml in analyzed samples
50	1.25	8.0→10.00	40	12.40
100	2.50	8.0→20.00	40	38.70
200	5.00	5.0→25.00	40	51.60
300	8.00	2.5→20.00	40	56.44

electrolyte at pH 4.0 containing DDP-OH, which has a strong UV absorbance. Compared with some other substances, preparation of DDP for electrolyte was quite time-consuming but accepted. As the method showed good results, the small disadvantage was evaluated to be acceptable.

The majority of imprecision in CE analysis has been reported to be related to variability in the volume of sample solution injected into the capillary due to the technical difficulties of nanoscale injections (in this method approximately 2 nl) [1,11].

This variation could be effectively eliminated by use of an appropriate internal standard. Inspired from structure of DAB (Fig. 1), different compounds were tested as candidates for internal standard for the method.

Piperidine was one of the candidates. Piperidine was well-separated from the DAB and readily soluble in both the separation electrolyte and sample dissolving solvent. Piperidine was also found to be suitably stable with an adequate UV response. Piperidine was selected as internal standard (Fig. 1).

The concentration of piperidine was chosen to be relatively high and close to DAB to minimize integrator errors.

3.2. Sample preparation development

The target of sample preparation was to reach a simple, practical, and almost identical procedure for API, solutions (dosing solutions and dissolution samples), and tablets (1.00, 12.5, 25, 50, 100, 125, 200, and 300 mg DAB).

DAB, (−)-tartrate was found to be very soluble and stable in water. The API was easily dissolved in water, and the internal standard was added. No sample preparation was preformed for dosing solutions and dissolution samples as DAB was already dissolved in these samples. First, these samples were diluted with water to 1000 μg/ml (if necessary) and then, added internal standard.

Experiments showed that extraction of DAB from the tablets were easy and reproducible using water as extraction media. Tablets were added a fixed volume of water, and DAB (as free base) was extracted by stirring the sample for approximately 60 min and centrifuging at 4000 rpm for 10 min. The end concentration of all samples was 1000 μg/ml of DAB before adding internal standard and dilution. Then, the sample was added internal standard and diluted. The final concentration of samples was approximately 40 μg/ml for DAB (as free base) and 30 μg/ml for the internal standard (piperidine).

The sample preparations were generally simple, practical, and almost identical for API, solutions and all tablets.

3.3. Validation

Validation was performed with respect to specificity and selectivity (matrix interference), linearity, range, accuracy, repeatability, intermediate precision, LOD, LOQ, robustness, and stability of standard and sample solutions.

The validation covered assay and dissolution methods for the API, dosing solution (1.00, 2.00, 4.00, 4.50, 5.00, 10.0, 20.0, 40.0, 47.0, and 50.0 mg/ml of DAB), dissolution samples (only for 50, 100, 200, and 300 mg tablets), and tablets (1.00, 12.5, 25, 50, 100, 125, 200, and 300 mg DAB).

3.3.1. Specificity (selectivity)

The method indicated a significant degree of specificity as good selectivity in separations.

The DAB and piperidine (internal standard) was well separated and easily quantified (Fig. 3).

The effect of placebo (matrix of tablet, dosing solution, and/or dissolution media) was also studied. The result showed that no placebo peaks occurred (Fig. 2).

The only possible impurity from the synthesis of the DAB API (a compound called PC 2507) were spiked to a sample containing DAB and piperidine and was injected in to the CE system. The separation was very good (Fig. 3).

The isolated degradation products from a forced degradation study was injected in order to demonstrate separation from possible degradation products. The result showed a very good separation of the degradation products and no interference with DAB peak (Fig. 4).

3.3.2. Linearity

The linearity of the method was tested by preparing a calibration curve for DAB with seven points. A total number of seven different dilutions of DAB,

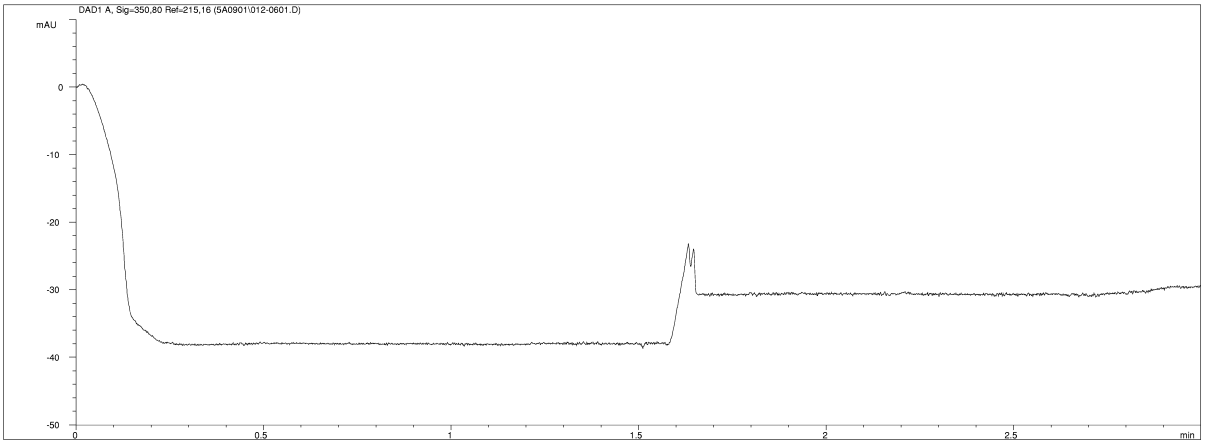

Fig. 2. Electropherogram of placebo (tablet, dosing solution, or dissolution media).

(−)-tartrate were prepared and injected in the CE system. The solutions were prepared in Milli-Q water containing the internal standard at its nominal concentration. The tested concentration range was from 29 to 54 μg/ml corresponding to approximately 70 to 130% of the nominal concentration of approximately 40 μg/ml. Each concentration level was injected three times.

The obtained data were used to evaluate the linearity using linear regression analysis. The area ratio of DAB to the internal standard was used for these analyses. The calculations were performed using Excel software.

The following linear regression parameters (linear regression analysis as $y = \beta x + \alpha$) were obtained for DAB: slope of the curve (β) = 0.0245266, intercept (α) = −0.0240636 and squared correlation coefficient (r^2) = 0.998.

During dissolution analyses, the internal standard was added to dissolution samples in different concentrations (approximately from 12 to 57 μg/ml). The linearity of the piperidine as internal standard

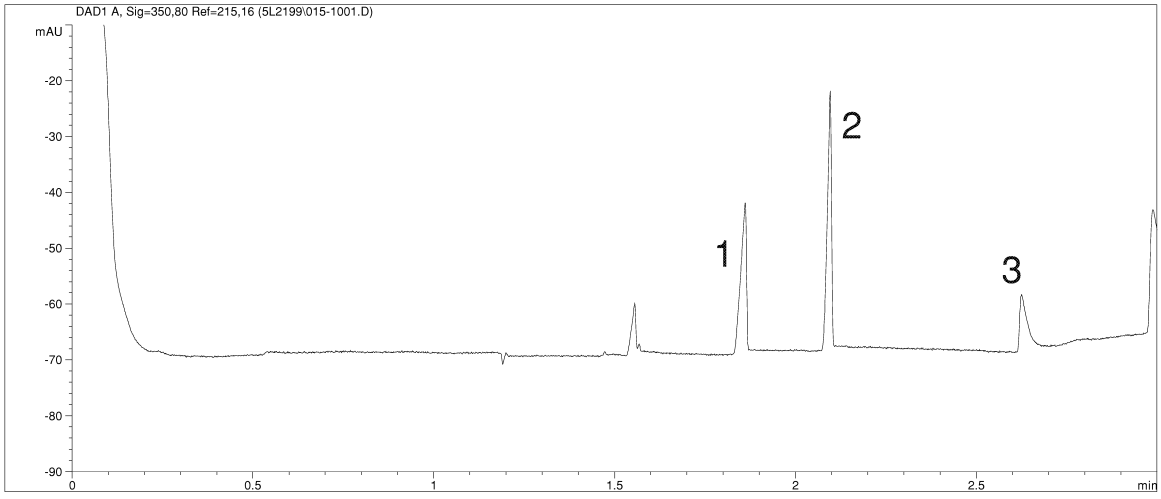

Fig. 3. Electropherogram of separation of the internal standard (1), DAB (2) and compound PC 2507 (3).

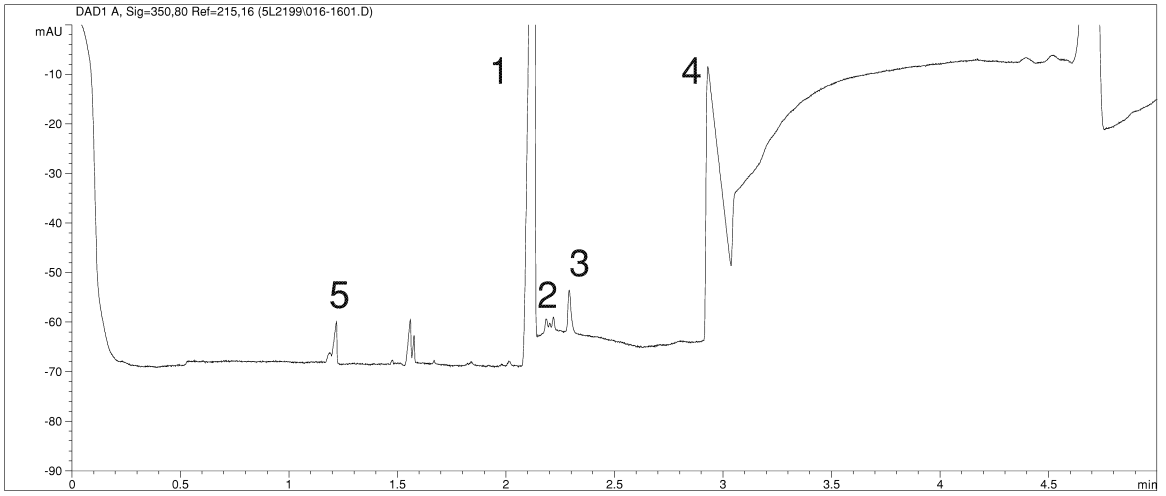

Fig. 4. Electropherogram of separation of DAB (1) and some degradation products (2, 3, 4 & 5).

was also tested by preparing a calibration curve with five points. A total number of five different dilutions of piperidine were prepared and injected in the CE system. The solutions were prepared in Milli-Q water. The tested concentration range was from 6.45 to 64.5 μg/ml corresponding to approximately 20–200% of the nominal concentration of approximately 30 μg/ml. Each concentration level was injected three times.

The obtained data were calculated using linear regression (method of least squares) on the area of piperidine. The calculations were performed using Excel software.

The following linear regression parameters (linear regression analysis as $y = \beta x + \alpha$) were obtained for piperidine: slope of the curve (β) = 0.00669, intercept (α) = 0.000812 and squared correlation coefficient (r^2) = 1.000.

The linearity was found to be good and was acceptable for DAB and piperidine.

3.3.3. Range

The tested concentration range for DAB was from 29 to 54 μg/ml corresponding to approximately 70–130% of the nominal concentration of 40 μg/ml.

The range of CE method (when injection volume was approximately 2 nl) for DAB was demonstrated to be from 58 to 108 fg.

The tested concentration range for piperidine was from 6.45 to 64.5 μg/ml corresponding to approximately 20–200% of the nominal concentration of 30 μg/ml.

The range of CE method (when injection volume was approximately 2 nl) for piperidine were demonstrated to be from 13 to 129 fg.

3.3.4. Accuracy

The accuracy of the method was demonstrated as percent recovery in three sample concentration levels far from each other and with three points at each level in order to reach a general picture of the method. The recovery was calculated using a standard solution at each level. The solutions were prepared using standard solutions and the placebo.

The recovery of the DAB was found to be between 99 and 101% (RSD = 0.8%) at 0.5 mg/ml level sample, 99 and 103% (RSD = 1.8%) at 10 mg/ml level sample and 98 and 102% (RSD = 2.2%) at 75 mg/ml level sample.

The accuracy results as percent recovery were found to be good and satisfactory for the method.

3.3.5. Repeatability and intermediate precision

Precision of the method was tested in two steps, namely the repeatability and intermediate precision.

The repeatability study was designed as follows: one sample solution was prepared at three con-

Table 3
Repeatability of the CE method in CE systems A and B

Sample concentration	Results (1 mg/ml)		Results (10 mg/ml)		Results (48 mg/ml)	
	A	B	A	B	A	B
$\bar{x}$	1.00	1.00	10.0	10.1	47.4	47.4
SD	0.0039	0.0064	0.0618	0.0652	0.2246	0.4229
RSD (%)	0.39	0.64	0.62	0.65	0.47	0.89
95% CI	±0.010	±0.016	±0.159	±0.168	±0.577	±1.087
Repeatability	0.53%		0.63%		0.71%	

centration levels (1.0, 10, and 48 mg/ml). Then, sample solutions were added internal standard and at the same time, diluted to test concentration (40 μg DAB as free base/ml). Six single determinations per solution (split in six portions/vial) were preformed on two CE systems.

The repeatability of the method was demonstrated by the mean value results, relative standard deviations of the determinations, and the confidence limits (95%) (Table 3).

The repeatability was demonstrated as a pooled RSD for the determinations by CE A and B. The injection repeatability was 0.53, 0.63, and 0.71% for the three analysed samples. An injection repeatability of approx. 0.6% was certainly acceptable.

The same repeatability data were calculated only using the DAB signal (peak area) without making correction with the internal standard. These results were given as corrected areas of the diluted solutions (Table 4).

The repeatability was also acceptable without taking the internal standard into calculation of the results. A larger relative standard deviation was observed for the highest concentration sample when not performing the calculations relative to the internal standard. This was in good agreement with the general concept that in CE, the use of an internal standard increases the precision by compensating for variability in injection volume [1,12–16].

These results also showed that the variation (RSD) in quantification analysis for the method was generally satisfactory, but becomes even better and more precise, when an internal standard is used.

The intermediate precision of the method was estimated by a reproducibility study between two laboratories.

Three different samples were prepared as originally 1 mg/ml (with placebo), 48 mg/ml (with placebo), and active pharmaceutical ingredients (API) as 1 mg/ml. Then, the samples were added internal standard and diluted. A single determination was preformed on samples with placebo and duplicate determination was performed on the API sample.

Different combinations of lab/technicians, instrument, capillary, and days were used for the experiment. The experiments were split between a total of three technicians from two departments, and each technician performed the experiments on four different days. The experiments were split randomly

Table 4
Repeatability of the CE method (systems A and B) without using the internal standard in result calculation

Sample concentration	Results (1 mg/ml)		Results (10 mg/ml)		Results (48 mg/ml)	
	A	B	A	B	A	B
SD	0.0019	0.0015	0.0032	0.0020	0.0028	0.0046
RSD (%)	0.52	0.47	0.88	0.62	0.81	1.53
Repeatability	0.50%		0.78%		1.18%	

between four different CE instruments; five different capillaries, and a total of four different stock standard solutions were used. A total of 12 measurements were performed on each sample for this study (Table 5).

The reproducibility for both the active pharmaceutical ingredient and the products was found to be acceptable.

The total intermediate precision given as the obtained %RSD for the two laboratories has been calculated based on the above showed data. The results can be seen in Table 6.

The obtained intermediate precision of the method was satisfactory.

3.3.6. LOD and LOQ

For determination of LOD, solutions with low concentrations of DAB, (−)-tartrate were injected in order to find the concentration corresponding to a signal-to-noise ratio of 3:1 (as USP Pharmacopoeia definition) [17].

A 0.1998-μg DAB/ml showed the best results. In order to also mimic the normal CE quantification (assay), the solutions contained the internal standard (piperidine) at its normal concentration level. The concentration 0.2 μg DAB/ml corresponded to 0.50% (when analytical concentration is 40 μg DAB/ml) was accepted as the LOD, this corresponded to approximately 0.4 fg DAB, when injection volume is approximately 2 nl.

For determination of LOQ, solutions with low (but higher than LOD) concentrations of DAB, (−)-tartrate were injected in order to find the concentration corresponding to a signal-to-noise ratio of 10:1 (as USP Pharmacopoeia definition) [17].

A 0.9364-μg DAB/ml showed the best results. In order to also mimic the normal CE assay, the

Table 6
Intermediate precision for department A and department B

Test samples	Dept. A (%)	Dept. B (%)
API (as 1 mg/ml)	1.66	0.61
1 mg/ml sample test (product)	0.87	0.35
48 mg/ml sample test (product)	0.88	1.22

Table 5
Reproducibility study: intermediate precision of the CE method

Measurement No.	Analyst/ Dept.	CE instrument	Capillary No.	Results			
				API (%)		Product (1 mg/ml)	Product (48 mg/ml)
1	1/A	C	4	99.2	100.5	1.01	47.2
2		B	1	100.1	97.6	1.01	47.8
3		B	4	100.9	99.9	1.01	47.5
4		C	2	98.6	99.4	1.02	47.3
5	2/A	A	4	97.9	103.2	0.99	47.0
6		C	2	102.7	101.4	1.02	48.3
7		B	3	98.9	100.7	1.01	47.2
8		C	3	98.4	101.9	0.99	47.6
9	3/B	D	5	100.7	100.9	1.01	48.2
10		D	5	99.9	101.1	1.01	49.1
11		D	5	100.2	101.2	1.00	49.6
12		D	4	101.6	101.6	1.00	48.7
	$\bar{x}$			100.4		1.01	48.0
	SD			1.44		0.01	0.83
	RSD (%)			1.43		0.72	1.73
	95% CI			±3.0		±0.02	±1.8

solutions contained the internal standard (piperidine) at its normal concentration level. The concentration 1.0 μg DAB/ml corresponded to 2.5% (when analytical concentration is 40 μg DAB/ml) was accepted as the LOQ, this corresponded to approximately 2 fg, when injection volume is approximately 2 nl.

The repeatability at the quantification limit level was tested using two CE systems. Same solution was injected six times in each CE system. The repeatability was given by the %RSD. The repeatability for CE A was 7.7 and for CE B was 8.5.

The repeatability in the quantification limit range was acceptable. The LOD and LOQ of the method were accepted.

3.3.7. Robustness

The robustness of the method was examined during the development of the method and as a routine part of the validation. Parameters such as different capillary, different instruments, and electrolyte ion strength capacity were examined for the CE method. The resolution between piperidine and DAB were determined to evaluate the separation. For sample preparation, the effect of stirring time was examined.

Generally, the method showed a very good robustness. Resolution between the piperidine and DAB peaks was maintained through changes in the method conditions.

The study also showed that ion strength capacity of electrolyte was very good, but a time limited electrophoresis period of 100 min (over 30 injections) was chosen to reach the most robust results. Changes in up to 25% in stirring time for sample preparation showed no effect in results.

3.3.8. Stability of analytical solutions

The stability of stock standard solutions, standard solutions, sample solutions, and electrolyte was examined.

The stock standard solutions (DAB and piperidine) were found to be stable when stored in a tightly closed container in refrigerator (2–8 °C) for 30 days. The standard solutions (diluted from stock solution) containing DAB and piperidine were found to be stable when stored in a tightly closed container in refrigerator (2–8 °C) for 15 days. The sample solutions were found to be stable when stored in a tightly closed container in refrigerator (2–8 °C) for 7 days. The electrolyte was found to be stable when stored in a tightly closed container at refrigerator for 30 days.

Stability study of solutions at room temperature showed that solutions stored in room temperature only have the half stability time of the solutions, which have been stored in refrigerator (2–8 °C).

The conclusion was that all solutions must be stored in a tightly closed container in refrigerator (2–8 °C). The solutions could be stored in a short period of time (up to 5 h) in room temperature during analytical work.

4. Conclusion

A high-speed, selective, and robust capillary electrophoresis method with high capacity was developed and validated for determination of assay and dissolution analyses of 1,4-dideoxy-1,4-imino-D-arabinitol (also known as DAB), tartrate in active pharmaceutical ingredients (API), solutions (dosing solutions and dissolution samples) and tablets during preclinical and Phase I and II clinical studies.

Good and acceptable method performance was observed for all validation points. The validation showed that the variation (RSD) in quantification analysis for the method was very satisfactory but becomes even better and accurate, when an internal standard was used.

The experimental part of the precisions study showed good results but also found to be too complicated for this type studies.

In general, the results demonstrated that the indirect UV detection by CE using internal standard was very suitable for the applied analysis.

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