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Short communication

Profiling of levoamphetamine and related substances in dexamphetamine sulfate by capillary electrophoresis

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ABSTRACT

A capillary electrophoresis method for the simultaneous determination of the enantiomeric purity of dexamphetamine as well as the analysis of 1R,2S-(−)-norephedrine and 1S,2S-(+)-norpseudoephedrine as potential impurities has been developed and validated. Heptakis-(2,3-di-O-acetyl-6-O-sulfo)-β-cyclodextrin was chosen as chiral selector upon a screening of neutral and charged cyclodextrin derivatives. Separation of the analytes was achieved in a fused-silica capillary at 20 °C using an applied voltage of 25 kV. The optimized background electrolyte consisted of a 0.1 M sodium phosphate buffer, pH 2.5, containing 10 mg/ml of the cyclodextrin. The assay was linear in the range of 0.06–5.0% of the impurities based on a concentration of 2.0 mg/ml dexamphetamine sulfate in the sample solution. Analysis of commercial dexamphetamine sulfate samples revealed the presence of 3–4% of levoamphetamine while norephedrine or norpseudoephedrine could not be detected, indicating that the compound was prepared by fractionated crystallization of racemic amphetamine. Comparison with polarimetric measurements indicated that dexamphetamine with an enantiomeric excess as low as 80% still passes the pharmacopeial test of specific rotation while an amount of 0.06% of levoamphetamine can be detected by capillary electrophoresis.

1. Introduction

Dexamphetamine (dextroamphetamine, 2S-(+)-amphetamine, (S)-1-phenyl-2-propanamine, Fig. 1) is the dextrorotary (S)-enantiomer of the racemic drug amphetamine. Both, dexamphetamine and amphetamine are controlled substances in most European countries and the United States of America. Dexamphetamine is a central stimulant therapeutically used in the treatment of narcolepsy [1] and the attention deficit hyperactivity disorder (ADHD) of children [2]. The effect in ADHD is believed to be mediated via several mechanisms including the binding of the drug to the pre-synaptic dopamine transporter inducing a reversed transport process as well as stimulation of pre-synaptic inhibitory autoreceptors resulting in reduced activity in dopaminergic and noradrenergic pathways [3].

Dexamphetamine sulfate is described in monographs by the United States Pharmacopeia 32 (USP 32) [4] and the British Pharmacopoeia 2009 (BP 2009) [5]. Both pharmacopeias determine the stereoisomeric purity of the drug by optical rotation. The USP states a specific rotation value $[\alpha]_D^{25} = 20–23.5°$ ($c = 4$ in water), the BP 2009 specifies $[\alpha]_D^{25} = 19.5–22°$ ($c = 8$ in water). In addition, the USP determines the chromatographic purity limiting individual impurities to 0.1% and the sum to 0.5%. No impurities are specified. The BP 2009 does not prescribe such a test. As the optical rotation is rather low and, therefore, prone to low sensitivity with regard to the detection of the R-enantiomer several other techniques have been applied to the determination of the enantiomeric purity of dexamphetamine drug substance. These include enhancement of the optical rotation upon derivatization [6], NMR using europium shift reagents [7], complexation by heptakis-(2,3-di-O-acetyl)-β-cyclodextrin [8,9] or derivatization with 1R-(−)-myrtenal [10] as well as chromatographic techniques [11–13].

Capillary electrophoresis (CE) has been recognized as a suitable technique for the determination of the stereoisomeric purity of drugs [14–22] as well as the analysis of related substances [16,17,19,23]. Moreover, it has been demonstrated that CE can be used to determine chiral impurities as well as (achiral) related substances simultaneously [24,25]. Many publications have described the enantioseparation of amphetamine and other phenylalkylamines by CE, for example [26–33]. Only one study has employed CE for the determination of the enantiomeric composition of amphetamine in comparison to NMR upon complexation

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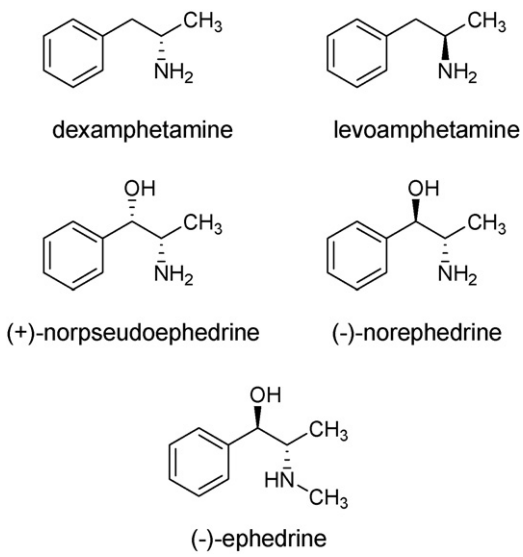

Fig. 1. Structures of dexamphetamine, levoamphetamine, 1R,2S-(−)-norephedrine, 1S,2S-(+)-norpseudoephedrine and the internal standard 1R,2S-(−)-ephedrine.

with heptakis-(2,3-di-O-acetyl)-β-cyclodextrin [9]. No study has attempted the simultaneous determination of related substances and the enantiomeric purity of dexamphetamine. In the present study 1R,2S-(−)-norephedrine and 1S,2S-(+)-norpseudoephedrine were studied as related substances because dexamphetamine can be prepared from these natural compounds [34,35].

2. Experimental

2.1. Chemicals

All chemicals were of analytical grade. Dexamphetamine sulfate and 1R,2S-(−)-norephedrine hydrochloride were from Fagron GmbH (Barsbüttel, Germany), racemic amphetamine sulfate and 1R,2S-(−)-ephedrine hydrochloride were from Sigma–Aldrich Chemie GmbH (Deisenhofen, Germany), and 1S,2S-(+)-norpseudoephedrine hydrochloride was from Hänseler AG (Herisau, Switzerland). Heptakis-(2,3-di-O-acetyl-6-O-sulfo)-β-cyclodextrin (HDAS-β-CD) was obtained from Regis Technologies (Norton Grove, IL, USA). Sodium hydroxide solution was from Fisher Scientific (Schwerte, Germany) and phosphoric acid was from Carl Roth GmbH (Karlsruhe, Germany). All buffers and solutions were prepared in deionized, double-distilled water.

2.2. Instrumentation

All analyses were carried out on a BioFocus 3000 instrument (Biorad, Munich, Germany) equipped with a diode array detector using 50 μm I.D., 375 μm O.D. fused-silica capillaries (BGB Analytik, Schloßböckelheim, Germany). The total length of the capillary was 51 cm with an effective length of 46.5 cm. Detection was carried out at 205 nm. The optimized background electrolyte consisted of 0.1 M phosphate buffer, pH 2.5, prepared from 0.1 M phosphoric acid by adjusting the pH with 1 M sodium hydroxide solution. HDAS-β-CD at a concentration of 10 mg/ml was dissolved in the buffer after the adjustment of the pH. The capillary was operated at 20 °C. The applied voltage was 25 kV.

A new capillary was treated with 1 M sodium hydroxide for 10 min, 0.1 M sodium hydroxide for 20 min, 0.1 M phosphoric acid and water for 10 min each. At the beginning of each day, the capillary was rinsed with water, 0.1 M sodium hydroxide and 0.1 M phosphoric acid for 5 min each followed by flushing with water for 10 min. Between the injections, the capillary was washed subsequently with 0.1 M sodium hydroxide for 1 min and water for 2 min followed by a rinse with the background electrolyte for 5 min. At the end of the day, the capillary was flushed with water for 2 min, 0.1 M sodium hydroxide for 10 min, water for 1 min, 0.1 M phosphoric acid for 5 min and water for 10 min. Samples were introduced by hydrodynamic injection at 0.5 psi for 2 s.

Polarimetric measurements were carried out on a Polarotronic E instrument (Schmidt & Haensch, Berlin, Germany) at a wavelength of 589.3 nm using a 10 cm standard cuvette thermostated at 20 ± 1 °C.

2.3. Method validation

The assay was validated for concentrations corresponding to a range of 0.06–5.0% for levoamphetamine (1.2–100 μg/ml racemic amphetamine) and 0.06–5.0% of (−)-norephedrine and (+)-norpseudoephedrine (1.2–100 μg/ml) based on a final concentration of 2.0 mg/ml dexamphetamine sulfate. Method validation was conducted according to ICH guideline Q2(R1) [36] with regard to range, linearity, limit of detection and quantitation, and precision. Linearity was estimated by unweighted linear regression using the least square method. Detection and quantitation limit were based on a signal-to-noise ratio of 3:1 and 10:1, respectively. Precision was determined at a low concentration (0.1%) and a high concentration (3.0%). Intraday precision was calculated from six replicate injections on the same day while interday precision was based on six injections on three consecutive days.

3. Results and discussion

3.1. Method development

The CE separation of the amphetamine enantiomers has been reported in several studies using native cyclodextrins (CDs) as well as neutral and charged CD derivatives as chiral selectors [9,26–33]. Thus, an initial screening was performed in a 50 mM phosphate buffer, pH 2.5, evaluating β-CD, 2-hydroxypropyl-β-CD, 2,6-dimethyl-β-CD, 2,3,6-trimethyl-β-CD, carboxymethyl-β-CD, succinyl-β-CD, sulfated β-CD and heptakis-(2,3-di-O-acetyl-6-O-sulfo)-β-CD (HDAS-β-CD) as chiral selectors. Good resolution of $R_S > 2$ was obtained with sulfated β-CD and HDAS-β-CD at concentrations of 1–3 mg/ml. Subsequently, HDAS-β-CD was selected for method development due to better peak shape and the fact that the CD is a single isomer derivative so that batch to batch reproducibility will not affect the performance of the analytical method as it may be the case with randomly substituted CD derivatives. Increasing buffer pH to 3.5 and 6.5 led to shorter migration times but also poorer peak shape. Thus, pH 2.5 was considered optimal. Injecting dexamphetamine at a concentration of 2.0 mg/ml led to peak deformation of the later migrating levoamphetamine. Increasing the concentration of HDAS-β-CD to 10 mg/ml and the buffer concentration to 0.1 M resulted in acceptable peak shapes of all analytes with an analysis time of less than 15 min (Fig. 2A). Thus, the optimized background electrolyte consisted of a 0.1 M phosphate buffer, pH 2.5, containing 10 mg/ml of HDAS-β-CD. At an applied voltage of 25 kV a current of about 90 μA was observed which was considered acceptable.

3.2. Method validation

The optimized method was validated according to the ICH guideline Q2(R1) [36] with regard to linearity, range, limit of detection (LOD), limit of quantitation (LOQ) as well as intraday and interday precision. Because enantiomerically pure dexamphetamine was not available, method validation was performed

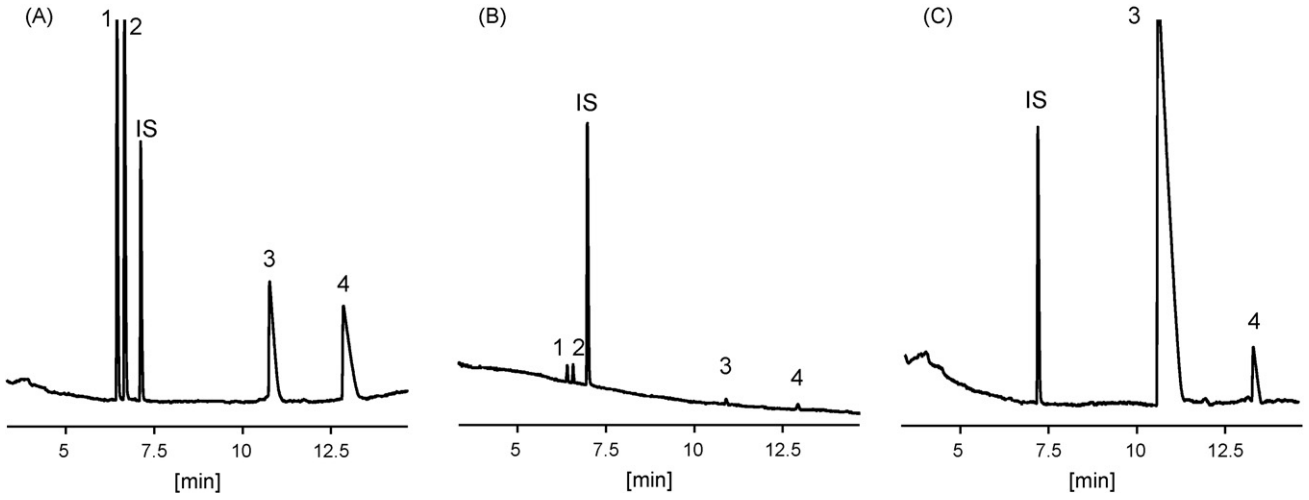

Fig. 2. Electropherograms of (A) separation of standards (75–144 μg/ml), (B) standards at the 0.1% (norpseudoephedrine and norephedrine) and 0.06% level (dexamphetamine and levoamphetamine) with respect to a concentration of 2 mg/ml dexamphetamine sulfate containing 75 μg/ml of ephedrine and (C) commercial sample of 2.0 mg/ml dexamphetamine sulfate containing 3.79% levoamphetamine. (1) 1S,2S-(+)-norpseudoephedrine, (2) 1R,2S-(−)-norephedrine, IS 1R,2S-(−)-ephedrine, (3) dexamphetamine, (4) levoamphetamine. Experimental conditions: 0.1 M sodium phosphate buffer, pH 2.5, containing 10 mg/ml HDAS-β-CD; 51/46.5 cm, 50 μM i.d. fused-silica capillary; 25 kV; 20 °C.

Table 1
Calibration data of levoamphetamine, 1S,2S-(+)-norpseudoephedrine and 1R,2S-(−)-norephedrine.

Compound	Range (%)[a]	Slope ± SD	Intercept ± SD	Correlation coefficient (r^2)	Intraday precision		Interday precision	
					0.1%	3.0%	0.1%	3.0%
1S,2S-(+)-Norpseudoephedrine	0.06–5.0	0.233 ± 0.002	−0.004 ± 0.006	0.9984	2.78	2.51	3.21	1.81
1R,2S-(−)-Norephedrine	0.06–5.0	0.233 ± 0.005	0.001 ± 0.014	0.9960	3.76	1.05	4.54	2.01
Levoamphetamine	0.06–5.0	0.219 ± 0.005	−0.004 ± 0.014	0.9954	6.54	3.07	6.70	5.33

[a] Corresponding to a concentration of 2 mg/ml dexamphetamine sulfate.

with racemic amphetamine. Levoamphetamine, norephedrine and norpseudoephedrine were analyzed over a range of 1.2–100 μg/ml corresponding to 0.06–5.0% of the impurities based on a concentration of 2.0 mg/ml dexamphetamine sulfate. Ephedrine at a concentration of 75 μg/ml was used as internal standard to compensate for injection errors and minor fluctuations of the migration time. Calibration data obtained by unweighted linear regression are summarized in Table 1. Correlation coefficients of at least 0.99 were observed indicating sufficient linearity in the investigated concentration range. The 95% confidence intervals of the *y*-intercepts included zero for all compounds so that a systematic error can be excluded. The LOQ estimated at a signal-to-noise ratio of 10 was 1.2 μg/ml (0.06%) for all compounds and the LOD corresponding to a signal-to-noise ratio of 3 was 0.4 μg/ml (0.02%) for norephedrine and norpseudoephedrine and 0.6 μg/ml (0.03%) for levoamphetamine. Fig. 2B shows an electropherogram of norephedrine and norpseudoephedrine at the 0.1% level as well as dexamphetamine and levoamphetamine at the 0.06% level. The RSD values of intraday and interday precision at 2 μg/ml (0.1%) and 60 μg/ml (3%) were below 7% for all compounds (Table 1).

3.3. Method application

The assay was subsequently applied for the analysis of commercial dexamphetamine sulfate drug substance. All samples were investigated at a concentration of 2.0 mg/ml containing 75 μg/ml of the internal standard. Neither norephedrine nor norpseudoephedrine could be detected in the samples. The content of levoamphetamine varied between 2.8 and 4.0% (Fig. 2C, Table 2). In addition to analysis via the peak area ratio, peak area normalization was also applied. Comparable concentrations of levoamphetamine were determined by both methods. The data indicate that the dexamphetamine sulfate batches investigated were not synthesized starting from the natural compounds norephedrine or norpseudoephedrine but were rather obtained by synthetic procedures leading to racemic amphetamine such as reductive amination starting from phenyl-2-propanone. The racemic amphetamine is subsequently resolved by fractionated crystallization using optically active acids such as (2S,3S)-(−)-tartaric acid [37]. Our data are in accordance with published data reporting amounts of levoamphetamine between 1.2 and 9.3% in dexamphetamine bulk drug and pharmaceutical preparations [11,12].

Only few of the above cited studies on the determination of the enantiomeric purity of dexamphetamine reported LOQ data. Using chiral europium shift reagents 5% of levoamphetamine could be determined by NMR [7]. Thunhorst et al. were able to determine 1.25% enantiomeric impurity in dexamphetamine by NMR using heptakis(2,3-di-O-acetyl)-β-CD as shift reagent [8,9]. The CD was also applied in an enantioselective CE assay but inconclusive data were obtained compared to the application in NMR [9]. A LOQ of 0.5% was reported for a HPLC assay employing a chiral stationary phase. The present CE assay allowed the determination of levoamphetamine down to the 0.06% level. Moreover, related substances can be simultaneously analyzed.

Table 2
Content of levoamphetamine in commercial samples of dexamphetamine drug substance (mean ± SD, $n = 3$).

Sample	[%] peak area ratio	[%] peak area normalization
1	3.79 ± 0.12	4.13 ± 0.22
2	2.79 ± 0.23	2.89 ± 0.13
3	3.97 ± 0.15	4.03 ± 0.05
4	2.88 ± 0.16	3.04 ± 0.35
5	3.26 ± 0.24	2.98 ± 0.26

Dexamphetamine sulfate sample 1 was also investigated by polarimetry according to the pharmacopeial tests of the USP 32 [4] and the BP 2009 [5]. A specific rotation $[\alpha]_D^{20} = 21.5 \pm 0.43^\circ$ ($n = 3$) was found. The addition of 2% and 4% racemic amphetamine resulted in values of $21.3 \pm 0.13^\circ$ and $21.1 \pm 0.17^\circ$, respectively. Considering the levoamphetamine content of sample 1 of 3.79% determined by CE, the optical rotation values correspond to an enantiomeric excess (ee) of 92.4%, 90.6% and 88.9%, respectively. From these values it can be estimated that a dexamphetamine sample with an ee as low as approximately 80%, i.e. containing 10% of levoamphetamine, would yield an $[\alpha]_D^{20}$ value of 20° still passing the test of specific optical rotation of the USP 32 ($[\alpha]_D^{25} = 20–23.5^\circ$) or the BP 2009 ($[\alpha]_D^{25} = 19.5–22^\circ$). This clearly indicates the limitations of the determination of the specific rotation of a dexamphetamine sample for the analysis of the stereochemical purity of the drug.

4. Conclusions

A CE method has been developed and validated for the simultaneous determination of some potential impurities of dexamphetamine as well as the stereochemical purity of dexamphetamine. The method allowed the determination of the impurities at the 0.06% level. While the related compounds could not be detected in commercial samples of dexamphetamine sulfate, a content of 3–4% of levoamphetamine was generally found. This indicated that the drug was obtained by fractionated crystallization of racemic amphetamine. Compared with the measurement of the optical rotation the stereoselective CE assay proved to be superior as a dexamphetamine sample with an enantiomeric excess of as low as 80% still met the criteria of the test of the optical rotation of the USP 32 and the BP 2009. As CE is capable to simultaneously analyze related substances and the stereochemical purity of drugs such methods may be preferable to enantioselective chromatographic methods or other methods reported in the literature.

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