DETERMINATION OF DEXTROMETHORPHAN HYDROBROMIDE IN PHARMACEUTICAL SAMPLES BY CAPILLARY ELECTROPHORESIS WITH CAPACITIVELY COUPLED CONTACTLESS CONDUCTIVITY DETECTION (CE-C⁴D)

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TÓM TẮT

XÁC ĐỊNH DEXTROMETHORPHAN HYDROBROMIDE TRONG MẪU DƯỢC PHẨM BẰNG PHƯƠNG PHÁP ĐIỆN DI MAO QUẢN SỬ DỤNG DETECTOR ĐỘ DẪN KHÔNG TIẾP XÚC (CE-C⁴D)

Phương pháp điện di mao quản sử dụng detector độ dẫn không tiếp xúc (CE-C⁴D) với ưu điểm hoạt động đơn giản và chi phí thấp đã được nghiên cứu nhằm xác định Dextromethorphan hydrobromid (DEX-HBr) trong mẫu dược phẩm. Các điều kiện phân tích gồm: dung dịch đệm điện ly Tris 30 mM/ His 10 mM/ Cyclodextrin 2 mM được điều chỉnh đến pH = 6,0 bằng acid acetic; mao quản sử dụng là silica (50 μm ID) với tổng chiều dài 50 cm (chiều dài hiệu dụng 30 cm); thế tách là 20 kV; bơm mẫu sử dụng kỹ thuật thủy động học kiểu xi phông ở độ cao 25 cm trong 30s. Giới hạn phát hiện đạt được là 3,0 mg/L. Hệ số tương quan tuyến tính của đường chuẩn là R² = 0,9989. Phương pháp có thể áp dụng để sàng lọc nhanh và định lượng DEX-HBr trong các mẫu dược phẩm (thuốc ho) có công bố sử dụng hoạt chất này.
Từ khóa: *Thuốc ho, CE-C⁴D, Dextromethorphan hydrobromide.*

1. INTRODUCTION

Dextromethorphan hydrobromide (DEX-HBr) is common in more than 150 over-the-counter cough and cold remedies in the United States [1], being classified as a safe and effective antitussive that has a central action on the cough center in the medulla. DEX-HBr (see **Figure 1**) is the hydrobromide salt form of dextromethorphan, a synthetic, methylated dextrorotary analogue of levorphanol, a substance related to codeine and a non-opioid derivate of morphine [2].
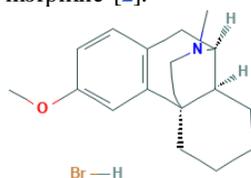
Figure 1. Chemical structure depiction of DEX-HBr

DEX-HBr exhibits antitussive activity and is devoid of analgesic or addictive property. This agent crosses the blood-brain-barrier and activates sigma opioid receptors on the cough center in the central nervous system, thereby suppressing the cough reflex [2]. It is a weakly basic compound (pK$_a$ of 9.3) [3], a widely used antitussive which typically appears in pharmaceutical compositions. At recommended therapeutic doses (20 – 30 mg), DEX-HBr has an excellent safety profile [4]; however, at doses associated with abuse (>100 mg), side effects will become apparent. This raises a big concern in developing countries in general, and in Vietnam in particular, where people can easily buy drugs for domestic use from retail pharmacies without medical

prescription [5]. Different methods have been reported for the determination of DEX-HBr in the bulk drug, in biological samples and in the dosage forms with other drugs in cough–cold products including: the first derivative technique UV spectrophotometry [6], High-performance liquid chromatography (HPLC) with UV detection [7, 8], gas chromatography coupled with mass spectrometry [9], and liquid chromatography coupled with scanning spectra focus detector [10].

The use of capillary electrophoresis (CE) in pharmaceutical analysis has benefits in terms of robustness and ruggedness, cost and time. The versatility of CE in the analysis of a wide array of pharmaceutically relevant analytes varying in polarity, size, and stereochemistry has been represented [11]. The high separation efficiencies obtained in CE are well suited for complex mixtures in which resolution of a number of peaks in a short time of analysis is desirable [11]. On the other hand, CE is an alternative for the development of simpler and less expensive analytical techniques.

Based on the need of a simple and low-cost method for determination of DEX-HBr as alternative to conventional HPLC, the purpose of this study is to employ a purpose-made CE instrument with capacitively coupled contactless conductivity detection (C^4D) as a simple and cost-effective approach for determination of DEX-HBr.

2. MATERIALS AND METHODS

2.1. Chemicals and materials

DEX-HBr were purchased at National Institute of Drug Quality Control (Vietnam), has a content of 99.9% $C_{18}H_{25}NO.HBr$, calculated on the anhydrous basis. The chemicals and reagents used were of pro analytical grade, including: L-histidine (His, $C_6H_9N_3O_2$), (Fluka, 99.5%), L-arginine (Arg, $C_6H_{14}N_4O_2$), (Fluka, 99.5%), trisaminomethane (Tris, $C_4H_{11}NO_3$), (Merck – Germany, 99%), cyclodextrin ($C_{42}H_{70}O_{35}$), (PA, Merck, Germany), acetic acid (Ace, CH_3COOH), (PA, Merck, Germany). The DEX-HBr containing tablet dosage forms were purchsed at pharmacies in Hanoi.

The stock solutions (1000 mg/L) of DEX-HBr was used for the preparation of standards of analytes. Background electrolyte (BGE) solutions were prepared by addition of the pre-selected acid (Ace) into a basic solution containing either Tris or His at desired concentrations and pH.

Fused silica capillaries of 50 µm ID and 365 µm OD were obtained from BGB Analytik AG (Böckten, Switzerland). Before use, the fused silica capillaries were pre-conditioned with 1 M NaOH for 10 min and deionized water for 10 min prior to flushing with the buffer. The capillaries were then used continuously for successive analyses. Deionized water purified was used for the preparation of all solutions and for sample dilution if required. pH values of solutions were controlled with an HI 2215 Hanna Instruments pH meter (Woonsocket, RI, USA).

2.2. Instrumentation

The CE-C^4D system was provided by the **3Sanalysis JSC.** (http://www.3sanalysis.vn/). Information about the specification of the equipment system can be found in the previous publication [12].
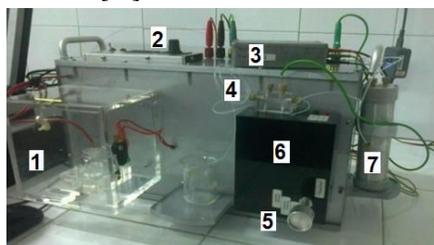

Figure 2. CE-C^4D instrument used in this study. (1) Safety cage for application of HV, (2) Electronic board for control of HV on/off and magnitude, which accommodates the HV adjustment trimmer and two digital screens for the monitoring of voltage and current values, (3) miniaturized HV – conductively coupled contactless conductivity detector, (4) flowcell interface housing one capillary end and the ground electrode, (5) rotary selector switch, (6) box containing solenoid valves, and (7) gas pressurized buffer container.

3. RESULTS AND DISCUSSION

3.1. Optimization of DEX-HBr analysis by

CE-C⁴D

Measurement conditions for DEX-HBr was investigated on CE-C⁴D instrument with the fussed silica capillary column, total length of 50 cm, internal diameter of 50 µm and voltage of +25 kV. The analytical conditions to be investigated include: composition and pH of buffer solution; the effective length of capillary, and sample injection conditions (injection time, injection height).

3.1.1. Effect of the buffer solutions

Buffer solutions are background electrolytes that ensure the movement of analytes in the capillary under application of voltages, therefore their compositions play the upmost important role on separation performance. In order to prevent high conductivity of buffer solution that may surpass the conductivity of analytes, the common buffers commonly used in CE-C⁴D constitute of low conductivity organic basic components like Arg, Tris, and His. They can be combined with acetic acid or ascorbic acid to obtain the desired pH value. In this experiment, the four buffer systems: Tris/His/Ace; Tris/His/Ace with cyclodextrin surfactant; Tris/His and Arg/Ace have been investigated for the determination of DEX-HBr. The electropherograms of DEX-HBr in these electrolyte solutions are shown in **Figure 3** below.
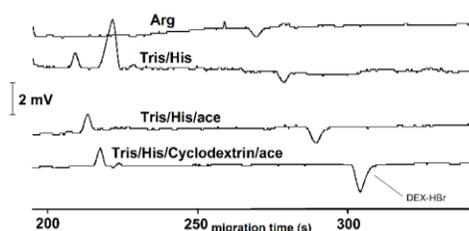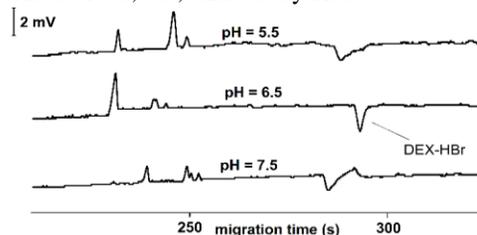
Figure 3. Electropherograms of 50 ppm DEX-HBr in different buffer solutions

According to **Figure 3**, in all buffer solutions, peaks of DEX-HBr appear downward, indicating that the conductivity of buffer was higher than that of the analyte. Best signal was obtained in the Tris/His/Cyclodextrin/Ace buffer solution with sharp, high and asymmetric peak and reasonable migration time of about 5 min. This buffer solution hence

was selected as the optimum background electrolyte for DEX-HBr determination.

CE technique separates substances based on their charges, therefore pH solution also plays an important role in the separation of analytes, since it determines the existence form of analytes and hence their charges. The investigation on optimum pH was carried out with 50 ppm DEX-HBr solution in the 10 mM His/Tris/Cyclodextrin buffers, adjusted to pH values of 5.5, 6.5, and 7.5 by Ace.

Figure 4. Electropherograms of 50 ppm DEX-HBr in Tris/His/Cyclodextrin/Ace buffer solution at different pHs, voltage 25 kV, injection time 25s

As shown in **Figure 4**, the DEX-HBr peak has poor signal at pH of 5.5 and 7.5. At pH of 6.5, peak of DEX-HBr appears well-proportioned with largest peak area and suitable analysis time. Hence, the pH of 6.5 was chosen for further investigation.

3.1.2. Effect of the effective length of capillary

Increasing the effective length can decrease the electric fields at a constant voltage, which increases the migration time and enhance the separation efficiency. When the effective lengths were varied as 25, 30, and 35 cm, the optimizing condition was gained at effective length of 30 cm, where we obtained the largest peak area of DEX-HBr, good baseline, and reasonable migration time (electropherograms not provided here).

3.1.3. Effect of sample injection

In our study, siphoning hydrodynamic method has been used to inject sample into the capillary. The injection time and injection height are two most important parameters that governs the injection volume and hence the

method sensitivity. The study on sample injection time was performed by varying injection times as: 20, 25, 30, and 35 s. The obtained electropherograms are shown in **Figure 5.**
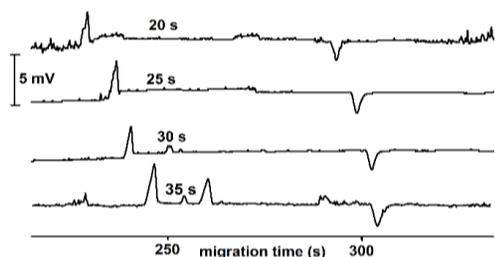
Figure 5. Electropherograms of 50 ppm DEX-HBr in Tris/His/Cyclodextrin/Ace buffer solution at different injection times

Figure 5 shows that with increasing the injection time from 20s to 35 s, the peak area also increases while the migration time does not change noticeably. The increase of the injection time results in the increase of the injected amount of sample into the capillary and hence the increase of the signal. However, too large injection volume may lead to peak broadening, thus, 30 s is chosen as suitable injection time for this study.

Similarly, the sample injection heights were also varied as 20, 25, and 30 cm, and best signal was gained at sample injection height of 25 cm, with largest peak area and highest peak resolution.

Optimum conditions for DEX-HBr determination are summarised in **Table 1** below:

*Table 1. The optimum conditions for DEX-HBr determination by CE-C^{4}D*

Buffer composition	30 mM Tris/10 mM His/2 mM Cyclodextrin, adjusted pH by Ace
pH	6.5
Injection time	30 s
Injection height	25 cm
Effective length	30 cm

3.2. Validation of the analytical method

3.2.1. Construction of the calibration curve

A series of standard solutions from 15 mM to 150 mM of DEX-HBr were prepared and injected to CE system at optimal conditions mentioned above. The average peak areas were used to make the calibration curves that represents the relationship between peak areas and concentrations of DEX-HBr. The equation of calibration curve of analyte was y = 0.0010x – 0.0008 with correlation coefficient of 0.9989. The good correlation coefficient ($R^2 \geq 0.99$) implies the linear relationship between peak area and concentration of DEX-HBr that can be used for determination of DEX-HBr in real samples. The calibration curve is given in **Figure 6**.
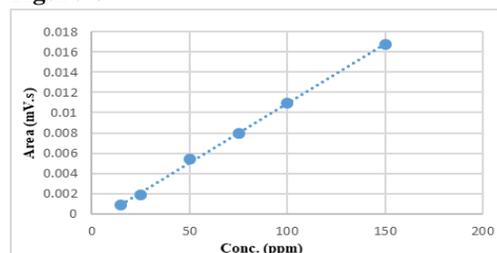
Figure 6. The calibration curve of DEX-HBr by CE-C4D method

2.2.2. Estimation of LOD and LOQ

To determine LOD, standard solutions are diluted to the lowest concentration at which the ratio of signal/noise equal to 3 (S/N=3). Generally, LOQ is defined as the ratio of signal/noise (S/N) equal to 10. The LOD and LOQ for DEX-HBr determination by CE-C^{4}D method was defined as 3 ppm and 10 ppm, respectively.

3.2.3. Evaluation of method accuracy and precision

The accuracy of method was evaluated through the method recovery using the standard addition method. The exact amounts of 30, 50 and 75 ppm, respectively, of standard solution was added to the sample matrix of the Dexpin's tablet. The recoveries of DEX-HBr at three concentration levels are in the range of 95.93% to 104.36%, meeting the standard of the Association of Official Analytical Chemists (AOAC) [13]. The relative standard deviation were less than 3.1% at all concentration levels, satisfying the requirement of AOAC [13] for ppm level determination.

3.3. Analysis of pharmaceutical samples

The developed method was applied to determine the content of DEX-HBr in cold-cough pharmaceutical samples, purchased from drug stores in Hanoi in July 2020. Samples were ground and mixed well. An exact amount

of 0.1 g sample was weighed and dissolved in 25 mL volumetric flask followed by filling up to the mark with deionized water. The obtained solution was ultrasound vibrated, filtered through a 0.45 µm filter, and diluted before analysis on CE-C^{4}D instrument.

Typical electropherograms of real samples are presented in **Figure 7**, showing clear DEX-HBr peaks without interference peaks. The same observation was obtained for the rest of tested samples. The contents of DEX-HBr measured by CE-C^{4}D in six drug samples are shown in Table 2 together with label content for comparison.
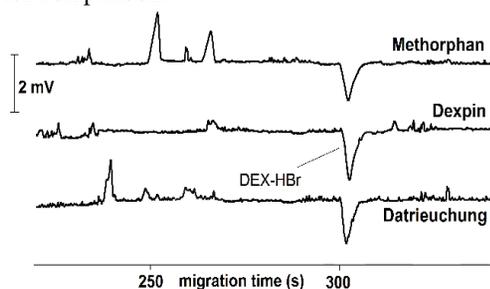
Figure 7. Electropherograms of several pharmaceutical samples

Table 2. The content of DEX-HBr in drug samples measured by CE-C^{4}D method

Drug samples	Measured by CE-C^{4}D (mg)	Label content (mg)	Deviation (%)
Methorphan (Traphaco - Vietnam)	9.32	10.00	- 6.8
Dexpin (Hataphar – Vietnam)	15.54	15.00	+ 3.6
Datrieuchung (Pharbaco – Vietnam)	9.34	10.00	- 6.6
Rhumenol Flu (Tenamyd – Vietnam)	14.54	15.00	- 3.1
SKDOL Flu (Phuong Dong – Vietnam)	14.37	15.00	- 4.2
Ameflu (OPV – Vietnam)	16.18	15.00	+ 7.8

From above results, contents of DEX-HBr in

drug samples differ from 3.1 to 7.8 % with contents provided by manufacturers in labels, indicating ensured quality of tested drugs and the capability to use our developed method for quantifying DEX-HBr in DEX-HBr containing drugs.

4. CONCLUSIONS

This study was successful in optimizing analytical conditions for the determination of DEX-HBr by CE-C4D instrument with low LOD of 3 ppm. The method has been validated and applied to analyze real pharmaceutical samples in Hanoi with the difference between measured contents and label claims from 3.1 ÷ 7.8 %. This work helps to promote the use of CE-C^{4}D as a straightforward and inexpensive solution for rapid and quantitative screening of various pharmaceutically active substances at local laboratories where only modest budgets and limited expertise are available.

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