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Pharmaceutical and Bio-analytical Applications of Ion Mobility Spectrometry for Determination of Clopidogrel (Plavix)

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Clopidogrel, with trade name plavix, is used effectively to reduce the incidence of ischaemic strokes, heart attacks and claudication. In this study, an ion mobility spectrometry (IMS) method was proposed for the clopidogrel determination in analytical and bio-analytical fields. Under experimental conditions, the analytical validation parameters of the proposed method were calculated and reported. The calibration graph was linear in the range of 30.0-2000.0 μg ml^{-1} for clopidogrel with two orders of magnitude and an appropriate fit of $R > 0.99$. The coefficient of variation (precision, n = 10) was found to be within 7.5-8.6%, and also the analyte concentrations of 3.0 and 10.0 μg ml^{-1} were obtained for the limits of detection and quantification, respectively. The developed IMS method was successfully applied to determine clopidogrel with an accurate recovery range (90.0-103.7%) in different matrices. The validation studies showed that the applied IMS was a simple, rapid, sensitive and reliable method for the clopidogrel analysis in the pharmaceutical and biological samples.

Keywords: Ion mobility spectrometry (IMS), Clopidogrel, Plavix, Pharmaceutical samples, Biological samples

INTRODUCTION

Clopidogrel (trade name: Plavix) is categorized in the thienopyridine class of antiplatelet agent that shows an inhibiting property on platelet s irreversibly by a receptor called $P2Y_{12}$. It is prescribed to decrease the risk of ischaemic strokes, heart attacks and claudication because of vascular problems like atherosclerosis. Clopidogrel is also used with aspirin for heart patients after the placement of a coronary artery stent. This drug is taken orally and its effects are started after about 2 h and continued for 5 days. The common side effects of clopidogrel are such as headache, nausea, easy bruising, and heartburn [1-6].

Because of the importance of clopidogrel, the various analytical methods including: spectrometry [7], chromatography [8-17], voltammetry [18], potentiometry [19] and electrophoresis [20] are seen in the literature for its accurate and sensitive determination in pharmaceutical and biological samples. Since the available methods usually suffer from expensive equipment and long analysis time, it is still necessary that the simple and rapid methods to be developed for quantitative analysis of clopidogrel.

Ion mobility spectrometry (IMS), an ion separation procedure on gas phase, is similar to time of flight mass spectrometry except that it operates under atmospheric pressure. IMS has been applied to determine an extensive range of compounds such as narcotics, drugs and pesticides. Corona discharge is an efficient ionization source in IMS. The ionization process of this source is based on the proton or electron affinity properties of analytes. High sensitivity, simplicity, and short response time are the major advantages of this technique [21-26].

The aim of present work was to develop and validate a simple, rapid and sensitive IMS method equipped with a positive corona discharge ionization source for determining clopidogrel. Solid phase extraction (SPE) and liquid-liquid extraction (LLE) techniques were employed to sample preparation. In accordance to the obtained results, the method could be successfully used and introduced for the clopidogrel analysis in pharmaceutical and biological

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samples.

EXPERIMENTAL

Apparatus

In this project, an ion mobility spectrometer equipped with continuous corona discharge as an ionization source was used. The instrument was constructed in the laboratory of Isfahan University of Technology (IUT, Iran). The main components of instrument are: an IMS cell, a needle and ring for producing the corona discharge, two high voltage power supplies, a pulse generator, a converter (analog to digital), and a computer to record and show spectra. A description of the instrument and its equipment has also been reported in Ref. [23]. The total peak areas of product ions over the acquisition time were obtained and gained using a laboratory made integrator program. The average of three experiments at each concentration level of analyte was measured and considered as the IMS signal intensity (response).

Chemicals and Materials

Clopidogrel powder (purity: >98%) was kindly supplied. The stock standard solution (100 μg ml^{-1}) was prepared by dissolving drug in distilled water. SPElC_{18} cartridge (Supelco Inc., 100 mg) was supplied from Sigma-Aldrich (St. Louis, Mo, USA). All chemicals and solvents were obtained from Fluka or Merck Co.

Sample Preparation

Pharmaceutical sample. A brand of Plavix (Cobel Darou, 75 mg) was prepared from a drugstore located in Yazd, Iran. Ten tablets of drug were weighed and crushed uniformly. The powdered drug (75 mg) was transferred into a volumetric flask and 10 ml of distilled water was also added to it. The mixture was sonicated for 5 min, and then it made up to 100 ml with distilled water. 1 μl of this solution was injected into IMS for the clopidogrel determination.

Human serum and urine samples. In the present study, typical biological fluids such as serum and urine were analyzed. Blank human serum and urine samples were prepared from healthy volunteers and stored in the refrigerator until analysis (taken from the Shahvali Hospital of IAU-Yazd).

A LLE technique was used for the sample preparation of serum. To one ml of spiked serum sample consisting of clopidogrel was added an equal volume of chloroform. Then, it was vortexed briefly and centrifuged at 4000 rpm for 5 min. The chloroform layer (consist of drug) was collected [27,28], and finally 1 μl of this solution directly introduced into injection port of IMS for drug analysis.

A SPE technique was used for the sample preparation of urine. First, 5 ml of this sample was diluted with 5 ml of distilled water and it was centrifuged at high speed for 5 min (14,000 rpm). A volume of 1.5 ml of solution was transferred to a glass tube and 2.0 ml of ammonium carbonate buffer solution (0.01 M, pH = 9.3) was added and centrifuged again at 14,000 rpm for 10 min. Then, it was subjected to the C_{18} cartridge column pretreated with 1.0 ml of methanol, 1.0 ml of wate, and 2.0 ml of ammonium carbonate buffer. After sample loading, the column was washed with 1.0 ml and 2.0 ml of distilled water and buffer solution, respectively. The clopidogrel was eluted with 1.0 ml of methanol [29], and finally 1 μl of this solution was injected into the IMS for drug analysis.

RESULTS AND DISCUSSION

Optimum Conditions for the IMS Analysis of Clopidogrel

To obtain the best results, the operational variables of IMS including: corona and drift voltages, injection port and cell (oven) temperatures, carrier and drift gas flow rates, and pulse width were investigated or adjusted. Among these, temperature (injection and cell) was an important and effective variable on the IMS analysis which should be optimized. The effect of this variable on IMS response was measured with following signal intensity of 300.0 μg ml^{-1} of analyte at the different values of temperature. The optimum injection temperature can differ with regard to the structure of compound, its stability and melting point. According to Fig. 1, increasing the temperature of injection port (>200 °C) increased the signal intensity of clopidogrel. Therefore, the temperature of 210 °C was selected as the optimum value of injection port. The cell temperature was also examined in the range of 160-200 °C (Fig. 1). As shown in Fig. 1, increasing the cell temperature could enhance the signal intensity and to amplify sensitivity.

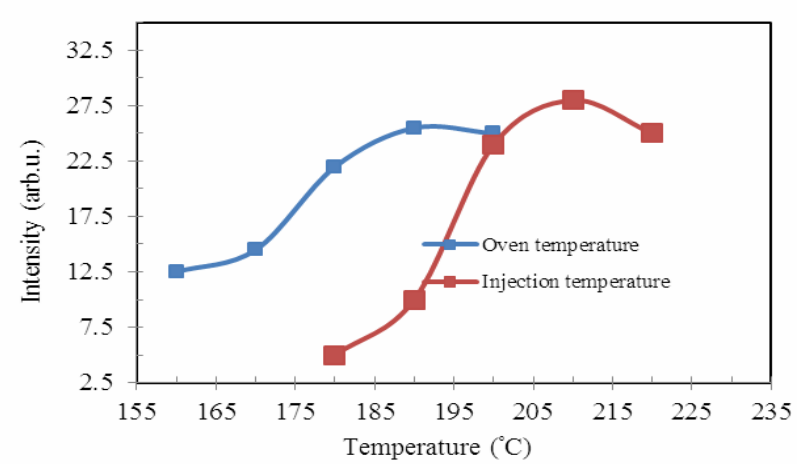

Fig. 1. The effects of injection temperature (180-220 °C) and cell temperature (160-200 °C) on the signal intensity (response) of clopidogrel. The other experimental variables were in accordance with Table 1.

Table 1. The IMS Eexperimental Conditions for the Clopidogrel Determination

Parameter	Setting
Corona voltage (V)	2300
Drift voltage (V)	6900
Drift gas flow rate (N_2, ml min^{-1})	600
Carrier gas flow rate (N_2, ml min^{-1})	300
Injection port temperature (°C)	210
IMS cell temperature (°C)	190
Pulse width (µs)	100

Thus, 190 °C was selected and applied as the optimum cell temperature. Other variables (voltages, flow rates and pulse width) were adjusted. The experimental conditions for determining of clopidogrel by IMS are summarized in Table 1.

The ion mobility spectrum obtained for clopidogrel in experimental conditions (Table 1) is displayed in Fig. 2. According to this figure, the spectrum showed only one peak at about 11.3 milliseconds in out of region where the reactant ions (NH_4^+, NO^+ and H_3O^+) appeared. Ion mobility spectrum of clopidogrel extracted from the tested samples exhibited that the sample preparation procedures provided clean extracts as any extra peaks were not observed in the region where the product ion of analyte appeared.

Analytical Parameters and Statistical Analysis of Data

Under the experimental conditions for IMS, a

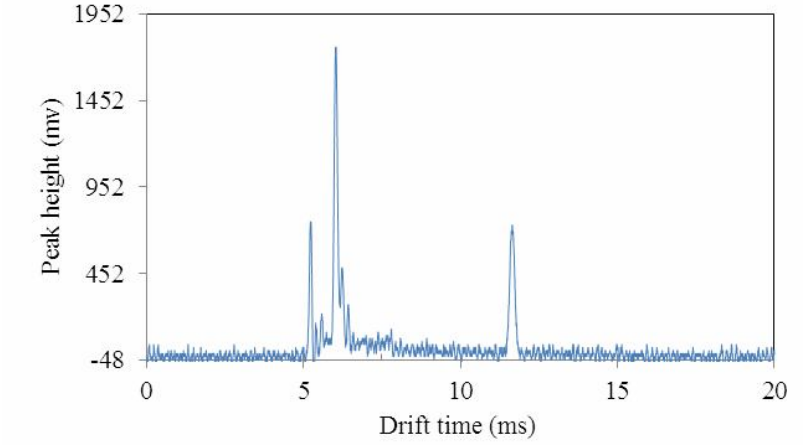

Fig. 2. The obtained ion mobility spectrum of clopidogrel. Conditions: Table 1.

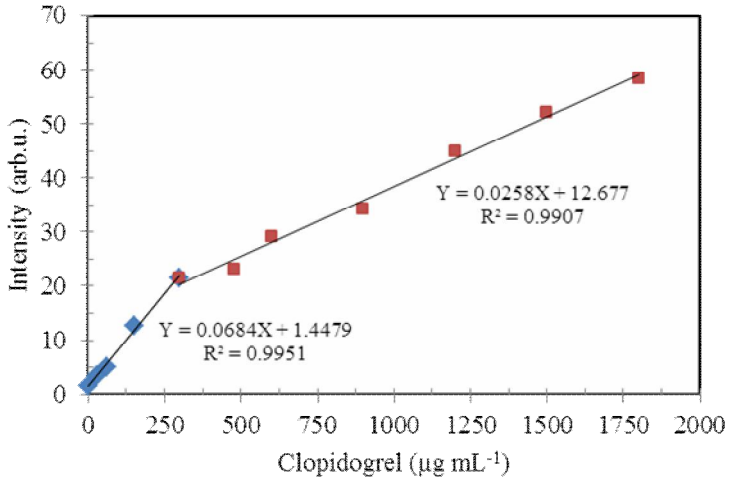

Fig. 3. Calibration graph of clopidogrel. Conditions: Table 1.

calibration graph was constructed by linear least squares regression of the ratio of analyte concentration to the peak area (Fig. 3). The linear dynamic range (LDR), slope and intercept of the regression equation are reported in Table 2. LDR was within 30.0 to 2000.0 µg ml^{-1} (2 orders of magnitude) with the correlation coefficient $R > 0.99$. The limits of detection (LOD) and quantification (LOQ) values were calculated using $3.3\ s_b/b$ and $10\ s_b/b$ that were found to be 3.0 and 10.0 µg ml^{-1}, respectively. Different concentrations (low and high levels) of clopidogrel were analyzed for the evaluation of intra-day precision. Eight replicates of each concentration were determined and reported for this purpose. The precision was satisfactory, with the RSD% ranging from 7.5-8.6%. The accuracy was also measured and explained as the relative error, so that the mean value of this parameter was less than 5%.

Table 3 presents the analytical parameters of the proposed IMS and the other existed analytical and bio-analytical methods for determining of clopidogrel. As shown, the LDR values of the proposed method were, and

Table 2. Analytical Parameters of the Clopidogrel Determination by IMS Method

Parameter	Results
Regression equation[a]	Y = 0.0684 X + 1.447
	Y = 0.0258 X + 12.677
LDR (μg ml^{-1})	30.0-2000.0 (R > 0.99)
LOD (μg ml^{-1})	3.0
LOQ (μg ml^{-1})	10.0
RSD%	8.6 and 7.5
	(600.0 and 1800.0, μg ml^{-1})
Accuracy (Error %)	<5

[a]Two-part equation. The experimental conditions: Table 1.

Table 3. Comparison of Analytical Parameters of the Proposed IMS Method with Various Methods for the Clopidogrel analysis

Method	LDR (μg ml^{-1})	LOD (μg ml^{-1})	LOQ (μg ml^{-1})	RSD (%)	Recovery (%)	Ref.
HPLC/MS/MS	$(10\text{-}10000) \times 10^{-6}$	-	10×10^{-6}	3.87	94.8-102.7	[11]
LC/MS/MS	$(1\text{-}800) \times 10^{-3}$		1×10^{-3}	10.9	88.5-101.3	[9]
HPLC-UV	10-60	3×10^{-4}	1×10^{-3}	< 2	99.7-100.0	[15]
TLC[a]	$(0.6\text{-}1.4) \times 10^{-3}$	0.024	0.079	1.06	90-110.0	[8]
Voltammetry	$(3.36\text{-}420) \times 10^{-6}$	8.40×10^{-6}	2.67×10^{-6}	2.4	99.1	[18]
Potentiometry	3.0×10^{-4}-3.0×10^{3}	1.0×10^{-4}	-	< 2	97.6-99.3	[19]
Spectrophotometry (UV-Vis)	40-65	0.4	2.0	1.12	99.5	[7]
Capillary electrophoresis	0.4-300	0.13	0.4	1.3	99.5-101.0	[20]
IMS	30.0-2000.0	3.0	10.0	7.5	90.0-103.7	This work

[a]TLC: Thin layer chromatography.

Table 4. The Application of IMS Method for the Determination of Clopidogrel in Pharmaceutical and Biological Samples

Serum[a]	Added ($\mu g\ ml^{-1}$)	Obtained ($\mu g\ ml^{-1}$)	Recovery (%)
1	405.0	420.0	103.7
2	780.0	720.0	92.3
Urine[b]	Added ($\mu g\ ml^{-1}$)	Obtained ($\mu g\ ml^{-1}$)	Recovery (%)
1	450.0	426.0	94.6
2	720.0	810.0	90.0
Tablet (Coble Darou)	Claim (mg)	Obtained (mg)	Recovery (%)
1	75.0	68.0	90.6

[a]LLE and [b]SPE was used for sample preparation. The experimental conditions: Table 1.

in some cases better than those of the other methods. The recovery results (accuracy) obtained in this work was within the range of those stated in literature. The LOD, LOQ and RSD values of the present work were comparable to the results reported in previous studies. Moreover, the developed IMS method allowed a simple and rapid assay of clopidogrel in various samples with a run time <1.0 min and a low-cost method respect to the other methods such as HPLC.

Applications to Pharmaceutical and Bio-analytical Studies

The IMS method was applied in the determination of clopidogrel at Plavix tablet (Cobl Darou, 75 mg). The obtained result for clopidogrel by the proposed method was in accordance to the labeled claim without any interference from excipients at tablet. The bio-analytical capability of the proposed method was also evaluated by determining of clopidogrel in serum and urine samples. For this purpose, the spiked biological samples at several levels were prepared. The sample preparation was performed by using LLE or SPE techniques and the extracted analytes were determined by the proposed IMS. The spiking recovery results were in the range of 90.0-103.7% (Table 4). This confirms that the developed method is adequate for the determination of clopidogrel in pharmacokinetic and biological studies.

CONCLUSIONS

This research describes the positive corona discharge IMS method for the determination of clopidogrel in pharmaceutical and biological samples. Analysis of tablet, serum and urine samples demonstrates the ability of the developed method to determine clopidogrel in real samples with acceptable accuracy and precision. The analytical validation parameters of drug analysis using IMS method are better than or comparable to those of other methods offered for this purpose. Although there are advantages for using other methods, the proposed IMS method is simple, rapid, and no need to the expensive equipment and dangerous solvents. The present study can develop the applications of IMS in pharmaceutical and bio-analytical fields.

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